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EMBRYOLOGICAL STAGES IN THE SEA LAMPREY AND EFFECTS OF TEMPERATURE ON DEVELOPMENT

By George W. Piavis

FISHERY BULLETIN 182
From Fishery Bulletin of the Fish and Wildlife Service
VOLUME 61

Published by the U.S. Fish and Wildlife Service • Washington • 1961
Printed at the U.S. Government Printing Office, Washington, D.C.

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington 25, D.C. - Price 30 cents

Library of Congress catalog card for the series, Fishery Bulletin of the Fish and Wildlife Series:

U.S. *Fish and Wildlife Service.*
Fishery bulletin. v. 1–
Washington, U.S. Govt. Print. Off., 1881–19

v. in illus., maps (part fold.) 23–28 cm.

Some vols. issued in the congressional series as Senate or House documents.
Bulletins composing v. 47– also numbered 1–
Title varies: v. 1–49, Bulletin.
Vols. 1–49 issued by Bureau of Fisheries (called Fish Commission, v. 1–23)

1. Fisheries—U. S. 2. Fish-culture—U. S. I. Title.

SH11.A25 639.206173 9–35239 rev 2*

Library of Congress [r55e¾]

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ABSTRACT

Early embryology of the sea lamprey has been subdivided into 19 stages. The stages are based largely on external morphology, behavior, and organ function. The holoblastic cleavage of sea lamprey eggs exhibited two types of third and fourth cleavages, equatorial and meridional. The open blastopore had an apparent migration over the surface of the embryo until it became the anus. Gastrulation resembles teleostean gastrulation in some characteristics and amphibian in others. Lamprey neurulation resembles teleostean neurulation more closely than it does amphibian.

Sea lamprey eggs were reared experimentally at 10 constant temperatures (at 5° intervals from 45° to 80° F., inclusive, and at 52.5° and 77.5° F.). No viable, burrowing larvae were produced at any temperature below 60° F. or above 70° F. Optimum temperature was 65° F. which yielded 78 percent survival to the burrowing stage; survival to the same stage was much lower at 60° F. (12 percent) and 70° F. (5 percent). The stage attained before all embryos were dead decreased as the temperatures were shifted in either direction from the 60°–70° F. interval. In general, developmental rate became faster, lengths of stages became shorter, and overlap between stages was lessened as temperature increased.

The evidence that sea lamprey eggs can develop successfully only within a limited temperature range suggests that unfavorable temperatures may account for the failure of certain apparently suitable streams to produce larval lampreys.

EMBRYOLOGICAL STAGES IN THE SEA LAMPREY AND EFFECTS OF TEMPERATURE ON DEVELOPMENT

By GEORGE W. PIAVIS, *Fishery Research Biologist*

he Great Lakes fisheries, the Nation's est source of fresh-water fishes for both com- cial and recreational fishing, have suffered etion of catch and the threat of disaster. danger has its origin in an increase in abund- of the sea lamprey, *Petromyzon marinus*, h is parisitic on and highly destructive of Major goals of the Great Lakes research ram of the U.S. Fish and Wildlife Service have to develop techniques for controlling this ace, to restore the Great Lakes fishery stocks n economically profitable level of abundance, to sustain them at that level.

thers have outlined in detail the history of the amprey within the St. Lawrence drainage and invasion of the upper Great Lakes: (Gage, ; Creaser, 1932; Hubbs and Pope, 1937; legate and Moffett, 1955). The sequence of invasion was summarized by Applegate (1950) llows: 1921, Lake Erie; 1934, Lake St. Clair; , Lake Huron; 1937, Lake Michigan; 1946, e Superior.

planning an attack against the ever-increas- numbers of sea lampreys, practically every e of their life history has been investigated pt that of early embryology. A search of the ature reveals little on the embryology of *narinus*, *Lampetra fluviatilis*, *L. planeri*, and *hyomyzon unicuspis*, the significant predators. less has been written concerning the other preys. Clear-cut stage designations are lack- for all lampreys. The usual embryological gnations for the common early stages of elopment are mentioned through the gastrula e, but even these lack clear definition.

order to portray accurately the embryology *. marinus*, a study was undertaken which had s objective the determination and definition he various stages of development.

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ery Bulletin 182. Approved for publication May 4, 1960.

In these studies, staging of lamprey development has been considered essential to a better understanding of the results of the series of experiments on effects of temperature. It was immediately obvious that differences in developmental time periods would result from variations in temperatures. In order to place the entire series of temperature experiments on a common basis, an accurate series of stages was of the utmost importance.

A second objective of this study was to determine the range of temperature for development of *P. marinus* eggs as well as their optimum developmental temperature.

This work began with a preliminary investigation conducted on a part-time basis during the summer of 1954. Intensive investigations were carried on throughout the spring and summer of 1955 when temperature experiments were undertaken. Confirmatory temperature experiments at 65° F. (18.4° C.) were conducted in the summer of 1956. These latter experiments also provided materials for normal staging.

This research was conducted as part of my graduate training at Duke University while employed at Hammond Bay as a fishery research biologist by the Great Lakes Biological Laboratory of the U.S. Fish and Wildlife Service.

Embryological studies and experimental work on living materials were conducted at Hammond Bay where the facilities of the sea lamprey research laboratory were placed at my disposal by Dr. James W. Moffett, Director of the Bureau's Great Lakes Biological Laboratories, and Dr. Vernon C. Applegate, Chief of the Hammond Bay Laboratory. Sectioning and statistical work were conducted at Duke University. The University also provided me with a refrigeration unit for use at Hammond Bay and the other facilities and materials necessary to the investigation.

Dr. Edward C. Horn, Duke University, knows of my appreciation for his many criticisms and patient guidance. I wish also to thank the staff

of the Hammond Bay Laboratory, and especially John Howell, for courtesies shown me and the time spent in my behalf.

STAGES IN NORMAL DEVELOPMENT

MATERIALS AND METHODS

Eggs of the sea lamprey were taken from mature, nest-building or spawning lampreys found in either the Trout River or the Ocqueoc River, both tributary to Lake Huron, Presque Isle County, Michigan.

Spawning lampreys were seized in a forked grip which placed the lamprey between the forefinger and the middle finger while the thumb anchored the animal. Capture by hand, in this way, minimized the loss of specimens by injury. The lampreys were kept in stream water throughout the transfer from the stream to the central collecting point, and to the laboratory where they were tempered and stripped in preparation for fertilization. To condition the eggs and sperm adequately to the test temperatures the lampreys were tempered in running lake water 2 to 3 hours prior to the initiation of the experiments.

Eggs were removed rapidly from female lampreys, held by two people. One held the anterior end by placing a thumb within the oral disc, thus taking advantage of the cusps to prevent slippage. The other held the tail by means of a pair of pliers. While the lamprey was thus outstretched, a transverse slit was made with scissors in the midventral body wall at a point behind the heart and the liver, i.e., at the anterior level of the ovaries. If the females were ripe, eggs began to extrude from this initial opening, whereupon the lamprey was slit to the vent by a rapid stroke with half-open scissors. Since the eggs are completely free in the coelom of a ripe female, they were allowed merely to flow into a 10-liter battery jar which contained a few liters of lake water from the trough where the animals were tempered. Any eggs entrapped within the coelom or folds of the ovary were removed rapidly by dipping the lamprey into the water with the slit open. This method of removing the eggs was quicker and far superior to the milking procedure used by McClure (1893) and others (including the author) because the time required to clear the female of eggs was limited only by the dexterity of the operators. Furthermore, the eggs are not distorted or damaged. Blood was not introduced into the fertilization jar since the slit produced no blood providing nei the liver nor the heart was pierced.

The males were grasped in the manner descr for the females. The individual who was hol the lamprey by the oral disc forced the milt f within the coelom through the genital papilla stream directed over the eggs in the battery Two males were used for each female; four m and two females constituted the usual batch.

Immediately after the addition of sperm, th was provided with an air bubbler and placed trough previously brought to the desired temp ture. The entire operation from stripping placing in the jar was completed within 60 seco Twenty to 30 minutes after fertilization the were washed. Washing was repeated at least more times during the first hour to insure comp removal of excess sperm. After the first hour eggs were apportioned among enamel pans x 12″ x 2″) or, on occasion, 4-inch glass bo These containers were covered with glass pl to minimize the accumulation of water-bo debris, and submerged. Circulation of w beneath the glass covers and over the develo embryos was insured by allowing a small are the pans and bowls to remain uncovered. C was taken not to crowd the eggs in the contain Preliminary work had shown that eggs arrange more than a single layer were highly suscept to attack by fungus.

Development of the embryos took place in pans or bowls which were held at the desired t perature in either constant-temperature trou or in 20-gallon aquariums. Each insulated tro measured 12 feet by 2 feet by 9 inches and c tained an inner-water chamber surrounded b 3-inch outer-water chamber. Spaces beneath metal divider provided free access between inner and outer chambers. Water within inner chamber was provided with air from c trollable bubblers.

The troughs were equipped with thermost cally controlled heating units and refrigerat units which provided temperature control wit ±0.5° F. The refrigeration tubing, the heat elements, and the thermostat bulbs lay within 3-inch outer chamber.

Water was circulated over the thermal units a continuously revolving 6-vane water w driven by a Ratiomotor. Since water flo freely between the outer and inner chambers,

of the thermal units was transmitted with ime lag to the inner chamber which held the ner of eggs.

t for the high temperatures was provided onwil circulators and heaters used in aquaria with lake water and provided with a con- le air bubbler. Bowls and pans were used tain the eggs within the aquaria as in the s. Air bubblers and water circulation by Bronwil circulators provided air for the ia. The circulators equipped with a contact- ostat and thermometer can be utilized for emperature setting from room temperature iling. In order to attain the maximum from this piece of apparatus the aquaria located within a cool room where it was ed that the ambient temperature would not ove the desired temperature. The temper- variation for the circulator was advertised as ° F. In actual practice, however, temper- variation could not be noticed. The appara- st described was utilized for experiments at ratures of 65° to 80° F.; the troughs were or temperatures from 45° to 70° F.

r to each experiment, troughs and aquaria washed, air-dried, and refilled with lake ; the thermostat was then set at the desired rature. Observations of the temperature inute intervals for a period of 4 to 8 hours ccasional readings in the remainder of a 24- nterval, assured stabilization at the correct rature. After the desired temperature was ished, Taylor thermographs and hourly gs of total-immersion thermometers (placed merged rubber stoppers grooved to receive gave a further check. The thermometers et in such a manner as to be readily visible ut handling. Thermographs were not used the circulator but the temperature was ed for 4 to 12 hours prior to the initiation experiment. Because of the small varia- n temperature delivered by the circulator, a ograph record was considered unnecessary when the ambient temperature was ex- l to rise above that desired. Furthermore, merged thermometer was compared period- with the contact-thermostat and thermom-

sampling was random throughout the experi- , and in general, the procedure varied only y from that outlined below. Fertilization was considered zero time; the first sample was taken 20 to 30 minutes after fertilization. Thereafter, samples were taken at the following hours: 1, 2, 3, 4, ___ 12, 14, 16, 18, 20, 24, 28, 32, 40, 48, ___ 72. After the 72d hour samples were taken at 12-hour intervals until the end of the experiment. In some of the longer experiments, samples were taken each 24 hours after about the 18th day. In addition to the samples taken during the run, all remaining eggs and larvae were kept as a final sample.

Samples of specimens were placed in Syracuse dishes and the gross morphological characteristics of the embryos were observed under a binocular dissecting microscope. The microscope was equipped with a calibrated ocular micrometer with which all measurements were made. Immediately after these observations all samples were placed in Smith's fixative[1] for 12 to 24 hours, washed in several changes of water during a 24-hour period, and preserved in a 4-percent solution of formalin. This method of fixation and preservation was most satisfactory as judged by the pliability of the heavily yolk-laden eggs after 3 years' preservation.

Specimens to be sectioned for microscopic examination were washed overnight in running tap water, stained in alum-cochineal 16 to 24 hours, then dehydrated and embedded in paraffin. Sections cut at 5 to 10 micra were mounted and counterstained in a 0.5-percent solution of fast green in 95-percent ethyl alcohol and covered permanently.

DESCRIPTION OF STAGES

The following description of stages was based on materials which were taken from all of the batches of the different temperature series; but greatest emphasis was placed on observations of the 65° F. batches. Most characteristics of embryos of the other batches were identical with those reared at 65° F. Those differences which were observed are pointed out in the discussion of the particular stage. The time intervals listed for each stage include the time between the first and last appearance of the stage in the samples reared at 65° F.

The end-points selected for each stage were established after numerous observations of both

[1] Solution *A*: Potassium bichromate 0.5 gm., water 87.5 cc. Solution *B*: Formalin 10 cc., glacial acetic acid 2.5 cc. Mix solutions *A* and *B* immediately before using.

living and preserved materials. It was considered essential to be able to recognize all preserved stages at a later time as well as living stages at the moment of collection.

In addition to the gross morphological end-points, histological examination was used on occasion to define the stage more critically. The criteria selected were such as to include also the physiological differences. Furthermore, since the status of an animal is evidenced in part through its activity and movements, natural movements or activity were incorporated as far as possible into the staging criteria.

The method of fixation and preservation proved to be suitable for recognition of practically all end-points. The normally transparent prolarvae [2] became opaque in Smith's fixative. When selected specimens were fixed and preserved in 4-percent formalin, however, retention of all pigmentation and transparency aided staging. The formalin, of course, hardened the yolk mass and the notochord enough to prevent the use of these specimens for histological preparations.

Stage 0: Ovulated but unfertilized egg

Animal-pole depression: Present but just visible.

Cellular areas: Nuclear; animal-hemisphere cytoplasm; vegetal-hemisphere cytoplasm.

Size: 1.0 ± 0.2 millimeters.

Ovulated eggs within the coelom are assigned this stage. These creamy-white eggs are surrounded by a relatively thin jelly coat which expands when the eggs are shed into water. The stickiness of this coat causes sand grains stirred up by spawners to adhere to the surface of the egg.

The surface of the unfertilized egg has a small depression in the egg membrane over the nucleus and extends into the nucleus proper. A demarcation separates the nucleus and the surrounding cytoplasm. One-third the distance down the animal-vegetal axis the cytoplasm contains another demarcation between the cytoplasm surrounding the nucleus and the remaining cytoplasm.

The egg of the sea lamprey is telolecithal in that the egg consists of a relatively large amount of yolk and the nucleus is located at the center of the animal hemisphere.

[2] Hubbs (1943) defined a prolarva as a "larva still bearing yolk." In the present work prolarva includes stages 14–17.

This stage is initiated when the eggs are ovu into the coelom and ends with fertilization.

Stage 1: Zygote (fig. 1) hours 0-2

Animal-pole depression: Increases in dia and depth; disappears within about 1 hour.

Cellular areas: Identical to stage 0.

Size: 1.0 ± 0.2 millimeters.

Fertilization membrane: Appears within minutes after fertilization; is retained th stage 13.

FIGURE 1.—Lateral view of stage 1, zygote, show cellular areas.

This stage extends from the time of fertiliz to the time when the fertilized egg begins to u go first cleavage. A meridional section fertilized egg (fig. 2) shows the yolk plat

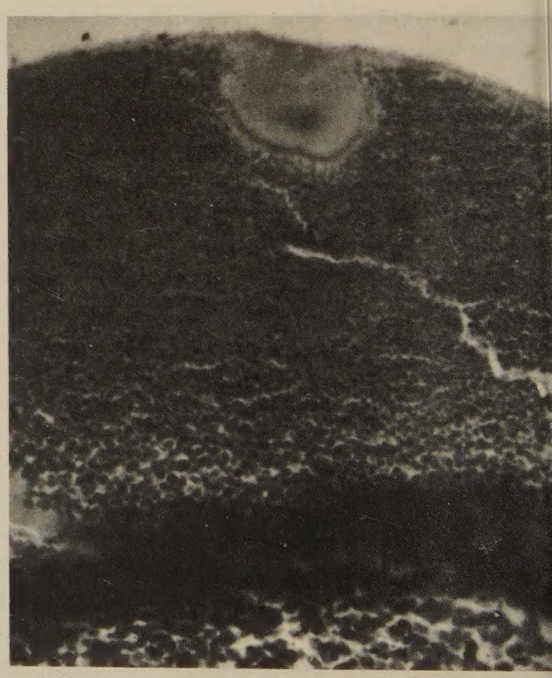


FIGURE 2.—Meridional section of stage 1 prior to f tion of fertilization membrane.

the cytoplasm surrounding the nucleus
e remaining cytoplasm. The yolk of the
and the vegetal areas differs in that the
latelets within the vitelline area are much
than those in the area around the nucleus.
depression at the animal pole seen in stage
sts through this stage. The first indication
ificant morphological change is a noticeable
ing of this depression which will remain
bout the first hour after fertilization.
ee distinct external areas indicate the
ce of the three internal areas: nucleus;
-pole cytoplasm; and vegetal-hemisphere
asm. Below the nucleus the cytoplasmic
demarcated by a band extending approxi-
one-third the distance down the animal-
l axis. The remainder of the egg consists
oplasm heavily laden with yolk. Each of
eas of the egg is visible both externally and
ally as early as the unfertilized egg and up
initiation of first cleavage.
rtly after the animal-pole depression deepens,
tilization membrane appears.

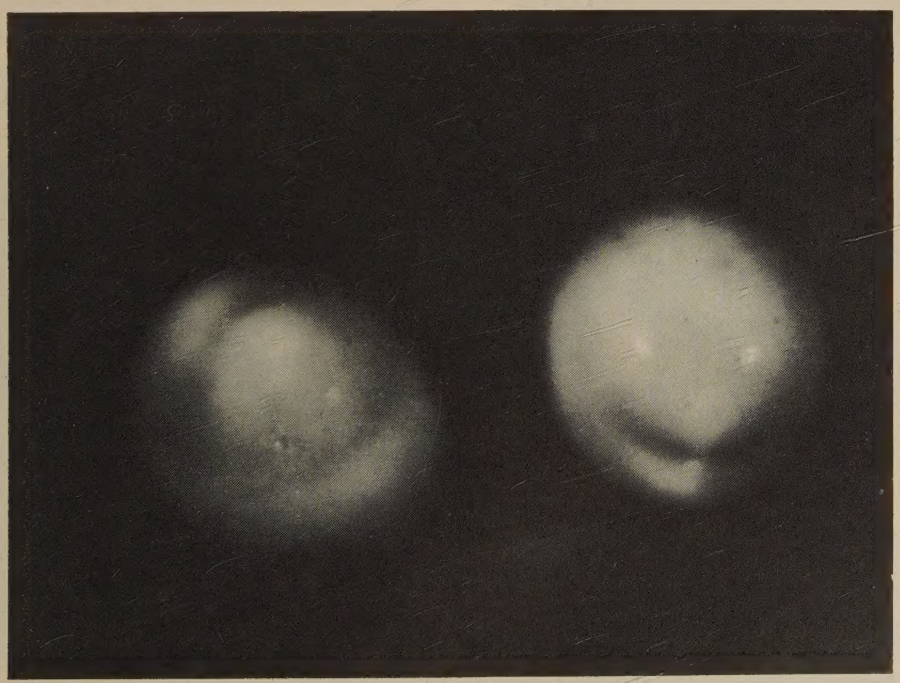
2: Two cells (fig. 3) hours 2-8

ular areas: Visible in the daughter cells.
: 1.0 ± 0.2 millimeters.

Cleavage: First furrow appears. Prominent peaks in daughter cells. Holoblastic. Completed within 5–6 hours.

The external topography of the 2-cell stage is comparable to that of the zygote in that the nuclear, and the animal- and vegetal-hemisphere cytoplasmic areas are readily visible in both daughter cells immediately after reconstitution of the nucleus (fig. 3).

This stage begins when the animal pole of the zygote begins to furrow and dimple in preparation for first cleavage, and ends at the beginning of the second cleavage furrow and dimple at the animal pole. Cleavage is total, usually slightly unequal (fig. 4) but occasionally (less than 1 percent) grossly unequal. As mentioned above, cleavage begins as a small furrow; a slight uprising of the cell membrane lateral to the furrow produces the dimpled effect. As cytokinesis progresses the cell membrane expands to a greater and greater extent while the fertilization membrane remains unchanged. The cell membrane continues to expand until the cell reaches the stage seen in figure 3, where the expanded cell membrane can be seen as twin peaks lateral to and above the furrow. As the cleavage furrow progresses meridionally over



e 3.—External views of stage 2, two cells, showing the expanded cell membrane (right side) and daughter cells showing the cellular areas.

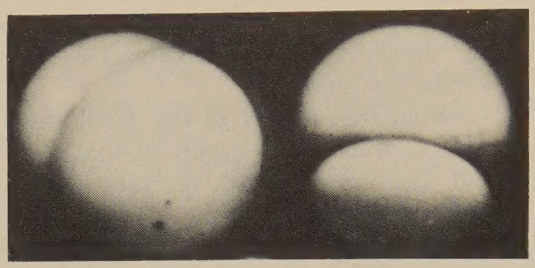


FIGURE 4.—External views of cleavage extremes of stage 2.

the cell, the cell membrane expands in advance of the progressing furrow.

Stage 3: Four cells (fig. 5) hours 8-11

Cellular areas: Visible in four daughter cells.
Size: 1.0±0.2 millimeters.

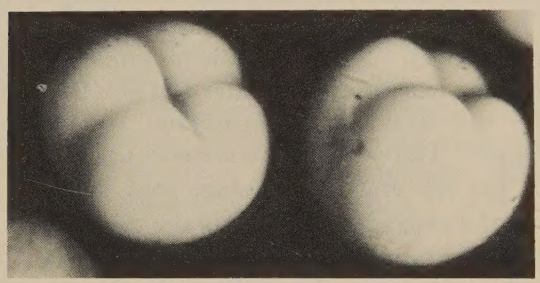


FIGURE 5.—Various external views of stage 3, four cells.

Cleavage: Second furrow appears: peak less prominent than in first division. Holobl Completed within 3 hours.

Despite cellular division the three distinct graphical areas are still discernible in each c four cells.

Stage 3 begins with the advent of the se cleavage furrow and dimple which starts in the same manner as the first; the furrow dimple are observed at the animal pole to e side of the first cleavage furrow and pro meridionally at right angles to the first cles furrow. As in stage 2, the expanding cell m branes are observed during cytokinesis.

The end-point of stage 3 is the appearan third cleavage furrow and dimple.

Stage 4: Eight cells (fig. 6) hours 10-15

Cellular areas: Visible in meridional cleavage.

Size: About 1.0 millimeter for equatorial sion. Meridional division increases equa diameter and shortens meridional diameter.

Cleavage: Two types; meridional or equat Holoblastic. Completed within 2 hours.

Stage 4 begins with the appearance of the cleavage furrow, which may be either a d

FIGURE 6.—Polar views of meridional and equatorial cleavages forming stage 4, eight cells.

ional furrow or a single equatorial division. meridional type was described by McClure) for *P. marinus* eggs held at 6°–8° C. (42.8°– F.) or at room temperature, whereas both of cleavage were found in this study. The orial cleavage was not mentioned by Mc-, although it predominated in the present in all experimental batches, regardless of erature.

bryos formed after meridional cleavage can tinguished by the flatness at the animal pole. nuclei are aligned four on each side of the leavage plane (fig. 6). In some embryos the entation cavity can be seen because the yo splits along the first cleavage plane.

e flatness of stage 4 embryos formed by lional divisions produces a large perivitelline which facilitates removal of the fertilization brane. The same operating space is not ntered again until stage 9.

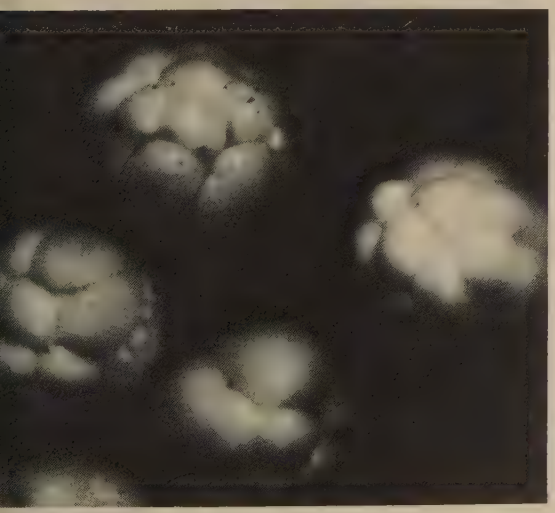
e embryos formed by equatorial divisions 4 micromeres resting upon 4 macromeres. hem, the space available for removal of the ization membrane is relatively small.

e end-point of this stage is the appearance of ourth cleavage furrow.

5: Sixteen cells (fig. 7) hours 13-15

llular areas: No longer recognizable from nal view.

e: 1.0 millimeter.



RE 7.—Several views of stage 5, sixteen cells, illus-ing differences in size of animal and vegetal cells.

Cleavage: Equatorial or meridional, depending upon type in stage 4. Pattern irregular. Completed within less than 2 hours.

Stage 5 begins with the appearance of the fourth cleavage, the plane of which varies according to the type of cleavage which formed stage 4; a third-stage equatorial division, near the animal pole, is followed by a fourth meridional division and vice versa. Cleavage irregularity becomes apparent during this stage; embryos composed of 9 to 16 cells are found and included in this stage.

The end-point of stage 5 is reached when the embryo is composed of 17 or more cells.

Stage 6: Thirty-two cells (fig. 8) hours 16-19

Size: 1.0 millimeter.

Cleavage: Random and indeterminate. Completed within 1 hour.

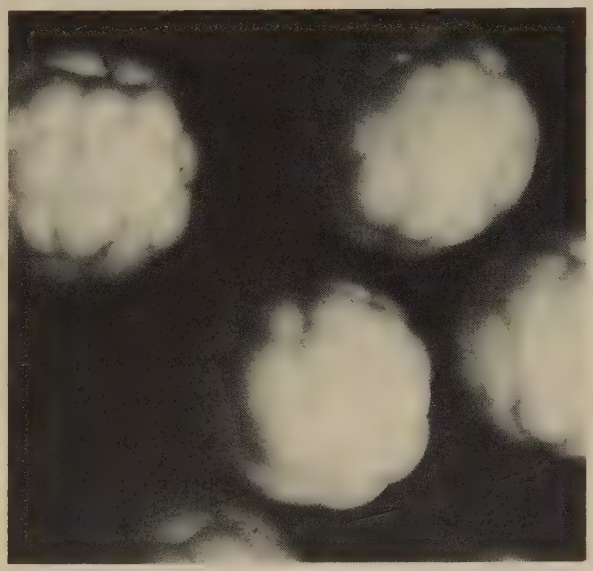


FIGURE 8.—Several polar views of stage 6, thirty-two cells.

Embryos were assigned to this stage when 17 to 32 cells were distinguishable. Cytokinesis at this point, however, became indeterminable so that the fifth cleavage appeared to take place at random. Cell counts were made on all embryos that could not be identified on the basis of relative cell sizes by comparison with both stages 5 and 7. When animal cells are compared with animal cells, and vegetal cells with vegetal cells, the cells of stage 6 embryos will be about one-half the size of stage 5 cells and approximately twice the size of stage 7 cells. The relative sizes of the animal-

and vegetal-cells can be ascertained in figure 9, a meridional section of stage 6.

The extent of the blastocoel can also be seen in figure 9. The roof of the blastocoel is composed of a single layer of relatively large animal cells whereas large vegetal cells extending from the vegetative pole to the blastocoel constitute the floor. The end-point of stage 6 is reached when the embryo has 32 cells.

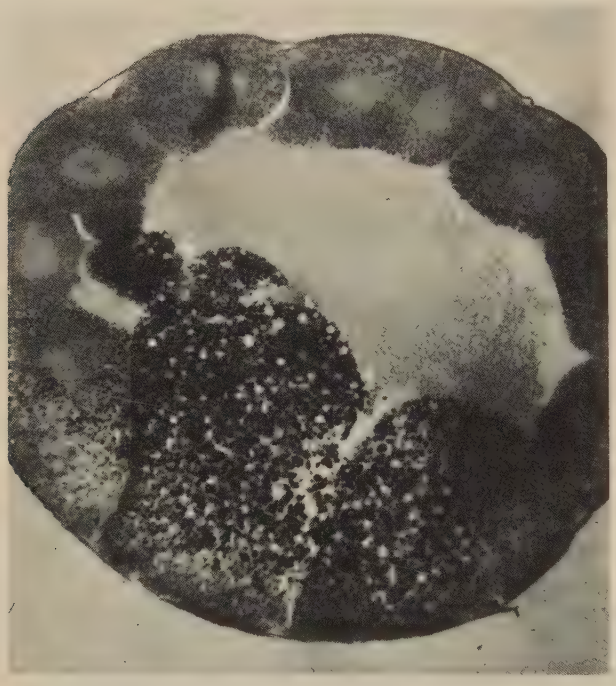


FIGURE 9.—Meridional section of stage 6.

Stage 7: Sixty-four cells (fig. 10) hours 19-24

Size: 1.0 millimeter.

Cleavage: Indeterminate.

Stage 7 is considered to have begun when the embryo has more than 32 cells. The cleavage continues indeterminable in this and later stages. Recognition of the stage becomes a matter of cell counts and comparison of cell sizes with those of the preceding and succeeding stages. A cursory count of the animal cells is made to get an approximate estimate of the stage to which the embryo should be assigned; the final assignment is based on a combination of cell size and cell count. As cell size diminishes the contour of the embryo becomes smoother. This change can be seen in a comparison of figures 8 and 10. The animal cells are still smaller than the vegetal cells, which have

FIGURE 10.—Polar and lateral views of beginning stag sixty-four cells.

since divided and are now only about twice size of the animal cells.

The end-point of stage 7 is reached when embryo has more than 64 cells. Division of animal cells along with division of the vegetal c can be taken as an approximate end-point.

Stage 8: Full blastula (fig. 11) hours 24-64

Size: 1.0 millimeter, increasing to 1.2–1.4 mi meters.

Cleavage: Furrows seen on individual cells.

Animal hemisphere: Becomes translucent.

Blastocoel: Visible through animal cells.

When the animal pole cells of stage 7 unde further cell division as evidenced by further red tion in cell size and the appearance of cleav furrows, the embryo is considered to have ente stage 8. The most striking external feature of t early phase of the stage is its contours (comp to stage 7) which become progressively smoot as the number of cell divisions increases and c size decreases.

In the final phase of this stage the volume of t embryo increases by some 3 to 5 times. Measu ments of living specimens made within a ca brated ocular micrometer at the initiation of a

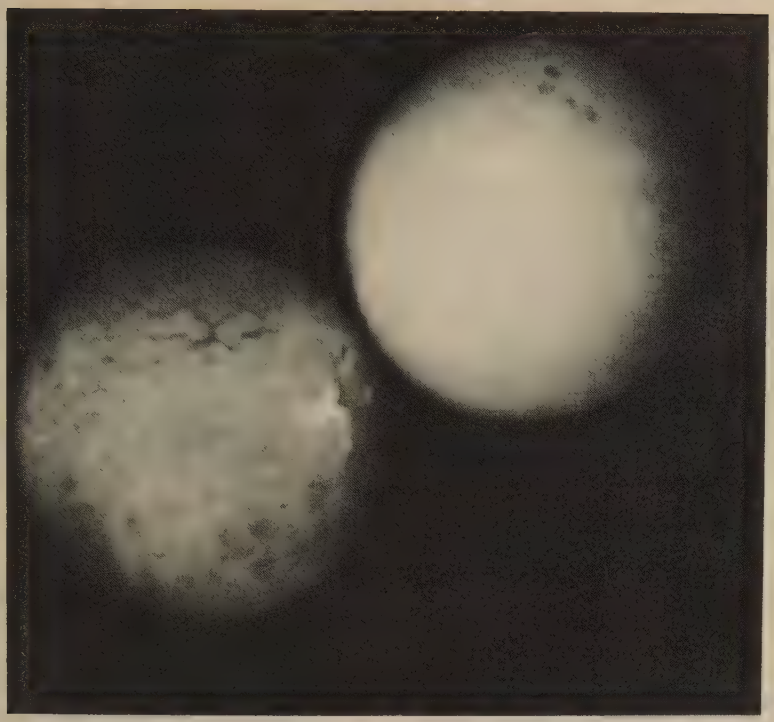


FIGURE 11.—Polar views of stage 8, full blastula, arranged to illustrate changes in cell size.

prior to this stage were consistently about 1.0 limeter across as viewed and measured in ical section perpendicular to the animal-etal axis as well as along that axis. Subse-nt measurements prior to the appearance of the sal lip of the blastopore, the end-point of ge 8, ranged from 1.2 to 1.4 millimeters. The rease in volume is accompanied by translucency he animal-pole cells so that the outline of the stocoel becomes visible through approximately -third the surface of the embryo. The size of animal cells does not change during the ansion process; the increase of the blastocoel icates that the expansion is due to blastocoel rgement. The blastocoel is at its greatest ume at the end of stage 8. The animal cells still about one-half the size of the vegetal cells. s size relation is maintained as long as the polizing animal cells can be compared externally n the underlying vegetal cells.

he end-point of stage 8 is the appearance of the topore.

ge 9: Gastrula (fig. 12) hours 64-104

ize: 1.2 to 1.4 millimeters decreasing to 1.0 imeter.

nimal hemisphere: Translucent to transparent to opaque. Opacity extends progressively forward from blastopore. Begins to flatten, forming neural plate.

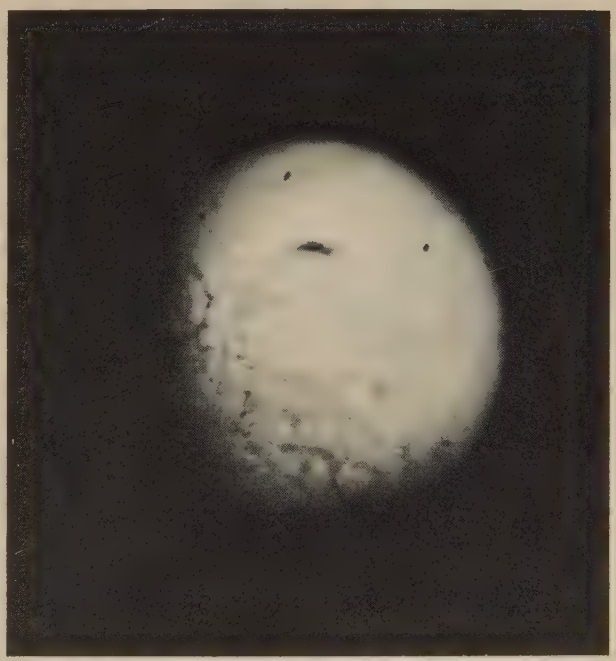


FIGURE 12.—Posterior view of stage 9, gastrula, illustrating the blastopore.

Blastocoel: Progressively obscured by opacity of animal hemisphere. Decreases in volume.

Blastopore: Forms as wide arched slit. Hooded as neural plate begins to form. Apparently migrates.

The translucency of the animal hemisphere of stage 8 changes to transparency after the appearance of the blastopore. Through the transparent animal hemisphere the underlying chorda-mesoderm is visible as it undergoes its morphogenetic movements. During this period the volume of the blastocoel decreases when the chorda-mesoderm reaches a position two-thirds the distance across the animal hemisphere. The most advanced portion of the chorda-mesoderm is in the mid-sagittal plane; the material in the parasagittal planes lags behind these more advanced cells. As this material progresses beneath the animal hemisphere, the dorsal lip of the blastopore progressively increases in thickness and begins an apparent migration from its original position at or near the overlap of animal and vegetal cells one-third the distance from the center of the animal hemisphere, toward the center of the animal hemisphere. As the chorda-mesoderm moves to its anteromost position in the embryo, the transparency of the animal hemisphere decreases, and the decrease in the volume of the embryo continues.

The typically circular blastopore of later stages is formed by progression of the animal cells in their epibolic movements to produce a changing pattern to the dorsal lip and, necessarily, the blastopore. At the time of the blastopore's inception the advancing margins of epibolizing cells beneath the blastopore approach each other laterally as a wide-open *V* with the apex at the center of the dorsal lip of the blastopore. As gastrulation progresses, these V-arranged margins close toward each other until only the vegetal cells below the blastopore remain uncovered. These vegetal cells are covered by epibolizing cells which move upward along the mid-sagittal plane (fig. 12).

As the chorda-mesoderm advances farther into the anterior portion of the embryo, the blastopore begins an apparent migration along the mid-sagittal plane from its original position to one located at the posterior limit of the mid-sagittal plane in stage 10. Histological comparisons between stages 8 and 9 indicate that this apparent movement resulted from reduction in the size of the blastocoel. At this time the dorsal region of the entire emb begins to flatten and to thicken from the dorsa of the blastopore to the anterior region.

Stage 9 is marked by the appearance of dorsal lip of the blastopore, a flat crescen shaped furrow within the overlapping line of animal cells epibolizing over the vegetal c Recognition of the stage depends upon loca the blastopore half-way beneath the embryo; observer must rotate the embryo to find it.

The end-point of stage 9 is reached when flattening process reaches the anterior end of embryo.

Stage 10: Neural plate and groove (fig. 13) days 4-5

Size: 1.1 to 1.3 millimeters.

Blastopore: Triangular to ovoid. Reaches dorsalmost point.

Neural tissue: Neural plate forms and thick Groove and folds form.

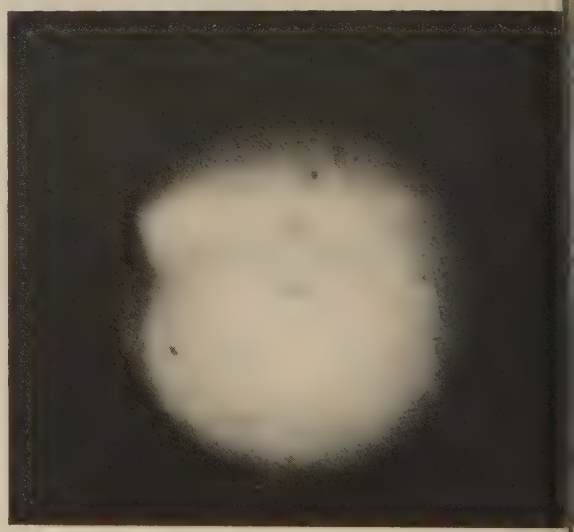
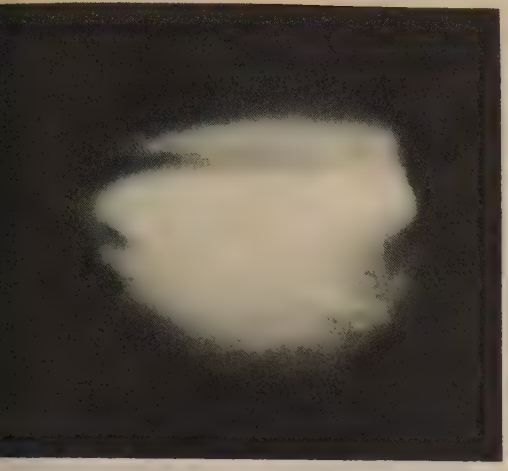
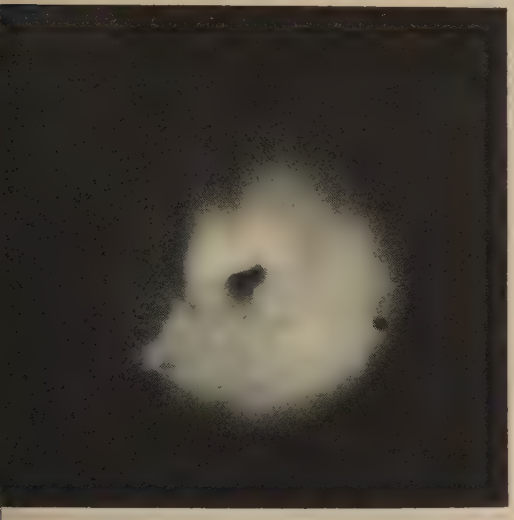


FIGURE 13.—Lateral view of stage 10, neural plate.

Stage 10 begins when the flattening of the dor ectoderm mentioned in stage 9 has reached anterior extremity of the embryo (fig. 14). At same time, the nearly completed blastopore near or at the dorsalmost point of the posterior a of the mid-sagittal plane of the embryo (fig. 1 Further thickening of the depth of the flatten area follows almost immediately after the fl tening of the dorsal ectoderm extends from t blastopore to the anterior region. These animal c are no longer transparent. The ovoid to triangu



RE 14.—Dorsal view of stage 10 illustrating neural groove.



RE 15.—Posterior view of stage 10 illustrating blastopore and neural plate with neural groove.

opore is now at the uppermost point of its rent migration along the mid-sagittal plane the flattened ectodermal cells (neural plate) t the height of their thickening. Practically ie vegetal cells are covered by the epibolizing al cells. Immediately after the neural plate ens, the central portion of the plate begins to a trough, producing a neural groove and stage (fig. 16). As the groove in the neural deepens the "folds" begin to approximate other and unite. The first actual union rs in the mid-dorsal region of the embryo. union is the end-point for stage 10.

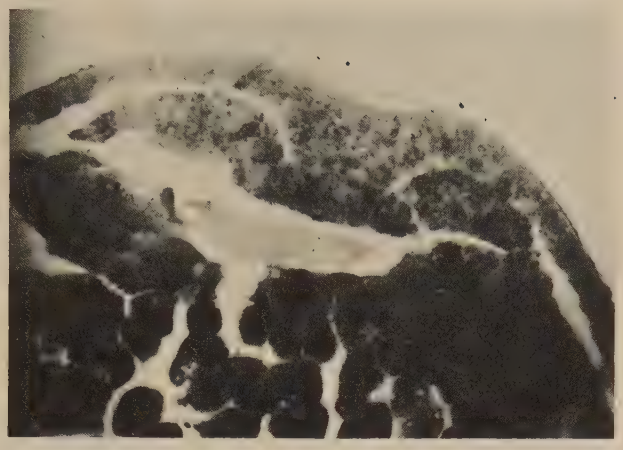


FIGURE 16.—Cross-section of stage 10

Stage 11: Neural rod (fig. 17) days 5-6

Size: 1.1 to 1.3 millimeters.

Blastopore: Circular. Apparent migration toward ventral surface of embryo.

FIGURE 17.—Oblique lateral view of stage 11, neural tube.

Neural tissue: Union of folds middorsally on embryo. Neural tube lacks neurocoel, and thus is a neural rod. Becomes prominent across dorsum of embryo; circumscribes approximately two-thirds of embryo.

In the late phase of this stage the dorsoposteriorly located, circular blastopore begins an apparent migration toward the ventral surface accompanied by a similar ventral movement of the prospective head. Toward the end of this stage the circular blastopore is at the ventralmost point of the posterior as contrasted with its

earlier position at the dorsalmost point of the posterior. These movements continue until the neural rod occupies approximately two-thirds of the circumference of the embryo.

The union of the folds marks the beginning of stage 11. The neural plate begins to round up and appears in external view to have become a neural tube with anterior and posterior neuropores. This appearance is a result of the apparently simultaneous, progressive union of the folds in anterior and posterior directions from the site of first union, the middorsum.

The external morphology of this stage is as deceptive as that of the preceding stage since histological examinations reveal that the neural tube does not possess a neurocoel; in reality the neural tube is a neural rod as described by Shipley (1885). In the anteromost region the neural rod seems to elevate from the surrounding and underlying tissue.

The end-point of this stage is reached when the anterior region is raised above the globular yolk mass.

Stage 12: Head (fig. 18) days 6-8

Size: 1.1 to 1.4 millimeters.

Blastopore: Below posteriormost point of neural rod, at ventral surface of embryo. Circular.

Neural tissue: Very prominent from head to blastopore. Neurocoel over presumptive pharynx.

Head: Elevated from yolk mass. Free length approximately 1.0 millimeter.

Yolk: Globular as in preceding stages.

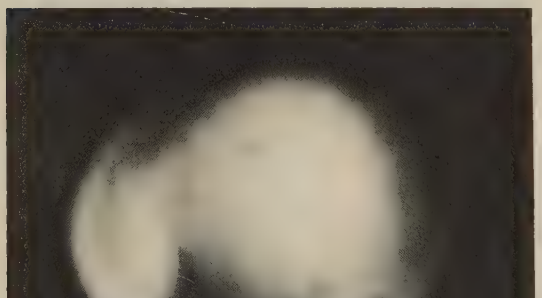


FIGURE 18.—Lateral view of stage 12, head.

Stomadaeum: Invagination begins.

Measurements of the embryo from the tip of head to the posteriormost point of the yolk along the neural rod gave a range from 1.0 millimeters when the head was just beginning form and 1.3 to 1.4 millimeters when the head fully formed and elevated from the yolk. Although sections show that some somites formed, they are not discernible externally.

The blastopore is below the posteriormost of the neural rod and faces directly ventrally during the initial period of this stage. When the head is fully formed, the blastopore moves anteriorly along the ventral surface toward the head.

Histological examination proves that the neural rod forms its neurocoel over the presumptive pharynx only. By this time the neural rod extends along approximately 75 percent of the periphery of the embryo (fig. 19). The rod is prominent and extends from the head to the blastopore.

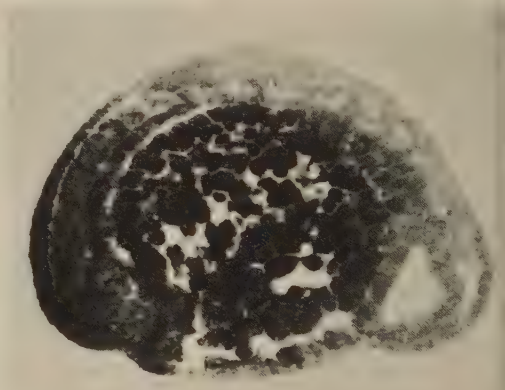


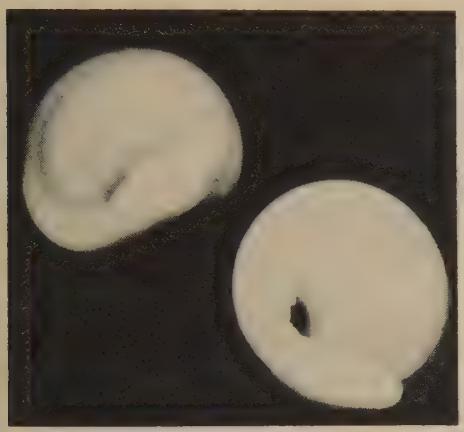
FIGURE 19.—Parasagittal section of stage 12.

Elongation takes place faster in the head region than in the tail region. The anterior part becomes well elevated and protrudes between the lateral swellings. The length of the head and the presumptive branchial region is about 1.0 millimeter at the end of the head stage. The appearance of a head on the exterior of the embryo marks the beginning of stage 12. This change is caused by the sudden increase in length of the neural rod during stage 11 and the sudden expansion of the presumptive pharyngeal cavity both dorsally and laterally to produce an upswelling of the respective head from its original position.

ne yolk mass retains the globular shape it had
ne preceding stage. The stomodaeal invagi-
on begins in the ventral portion between the
al swellings.
ne end-point of this stage is reached when the
region begins muscular activity.

e 13: Prehatching (fig. 20) days 8-12

ze: 1.4 to 2.5 millimeters.
lastopore: Circular. Much reduced. Lo-
d at anteriormost point of apparent migration.



IGURE 20.—Lateral view of stage 13, prehatching.

eural tissue: Neural rod now a true neural
. Prominent above somites from blastopore
ead.
ead: 2.0 millimeters.
olk: Obovate, blunt end posterior.
tomodaeum: Deepens and widens.
omites: 5 to 20; not easily distinguished
rnally.
ocomotion: First muscular activity. Move-
t of free embryo, head.
he length of the head region has increased
1 1.0 millimeter at the beginning to 2.0 milli-
ers at the end of this stage. Measurements
e taken from the tip of the head to the anterior-
t part of the yolk mass.
he blastopore, which is situated antero-
trally lies at its anteriormost position directly
osite the stomodaeal invagination.
tage 13 begins with the advent of muscular
vity. These movements initially are mere
ral flexions of the head and "neck" portion of
embryo; up-and-down activity is not apparent.
the embryo enlarges, muscular contraction
ides dorsoventral flexion and becomes un-

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dulating rather than wagging. With increase in in the embryo's size the perivitelline space becomes fully occupied, since the embryo, arranged in circular fashion within the membrane, begins to spiral upon itself. During this growth the above-mentioned movements become more forceful and more frequent.

Embryos that show movement but still have intact fertilization membranes are in stage 13. The endpoint of stage 13 comes when the head protrudes through the fertilization membrane to initiate hatching.

Stage 14: Hatching (fig. 21) days 10-13

Size: 3.0 to 5.0 millimeters.

Blastopore: Minute opening at apex of 90° ventral flexion of posterior. Dubious structure for staging.

FIGURE 21.—Lateral view of several prolarvae of stage 14, hatching, showing posterior curvature, somites, yolk mass, and condition of mouth and nostril.

Neural tissue: Neural tube still very prominent above somites.

Yolk (gut): Becomes slender and assumes a spatulate shape. Posterior region has 90° ventral flexion. Anteriormost portion greenish.

Stomodaeum: Deep pit located ventrally.

Somites: 18–20 to 30–35. Extend from neural tube to gut.

Locomotion: Hatching movements. Undulation of anterior body region only.

Nostril: Begins as single invagination in midventral line, anterior to the stomodaeum.

Transparency: First appearance of ectodermal transparency over pericardium. Extends posteriorly and anteriorly in later phases of this stage.

Circulatory system: Pericardium visible through transparent ectoderm. Straight tubular heart. Begins beating 40 times per minute.

Liver: First indication posterior to pericardium in anterior part of gut. Appears in late period of stage.

Stage 14 starts when the embryo breaks through the fertilization membrane. The constant movement observed in stage 13 finally becomes sufficiently strong to extrude the head through the membrane. Further activity enlarges the tear in the membrane and eventually leads to hatching.

The pericardial cavity appears as a ventral swelling approximately 1.0 millimeter posterior to the tip of the head. It is also present in late stage 13 but is not easily recognized in most embryos. Shortly after hatching the ectoderm overlying the pericardial region and the tissue beneath gradually become transparent. A short time later, the body anterior to the pericardial cavity becomes transparent.

Through these transparent tissues can be seen the straight tubular heart which begins to pulsate during the eleventh day at 40 beats per minute (at 65° F.). There is no sign of blood in the heart or anywhere in the prolarva.

The end-point of stage 14 is reached when melanophores appear on the embryo.

Stage 15: Pigmentation (fig. 22) days 13-16

Size: 5 to 6 millimeters.

Neural tissue: Brain and tube visible through ectoderm.

Gut: Spatulate, changing to cylindrical. V tral flexion of about 10° remains. Anterior greenish.

Stomodaeum (mouth): Transverse slit boun by thickened lips. Opens into oral cavity.

Somites: 35 to 50.

Locomotion: Undulation of entire body sligh restricted by yolk-filled gut in early stage. swimming movements in late period.

Nostril: Single, median at anteriormost point ventral surface.

Transparency: Extends anteriorly to branch region and posteriorly to about two-thirds length of the prolarva.

Circulatory system: Heart becomes S-shap Heart walls thicken. Grayish channel forms midventral gut and turns red as hemoglobin pears. Bilateral channels appear in 15th d Heart beat, 100 per minute.

Liver: Becomes larger and vascularized.

Pigmentation: First appears as bilateral melan phores dorsal to the midbrain.

The stomodaeal pit has become a slit openi into the anterior (oral) chamber of the phary which is separated from the posterior (pharynge chamber by the velum. The transverse slit bounded by a thickened ectodermal lip anterio and posteriorly. The pharynx has its full comp ment of 7 visceral pouches.

Deftness of swimming increases as the shape the yolk mass changes from spatulate to cylind cal. At the same time the tail straightens from ventral flexion. The prolarvae progress from t awkward movements of stage 14 to an undulati movement, the smoothness of which depends the amount of the ventral flexion of the tail.

The heart has enlarged and assumes an S-sha

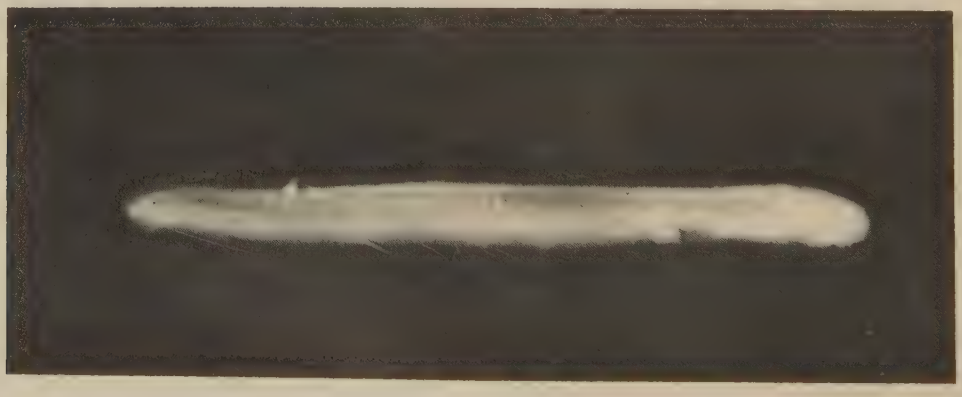


FIGURE 22.—Lateral view of stage 15, pigmentation, showing somites, pericardial area, and condition of yolk.

ıe auricle moves dorsal to the ventricle. Both ːular and ventricular walls begin to thicken ːemain transparent. The rate of heartbeat in- ses from the 40 per minute of stage 14 to 100 minute at the end of stage 15.

ːtween the 13th and the 14th days a grayish nel develops along the midventral line of the drical yolk mass. The channel extends from anterior portion to the midpoint of the yolk- l gut and within a short time turns red as oglobin is produced. During the 15th day eral channels appear along the midlateral line e yolk mass and then converge to form a single el to the heart.

age 15 is initiated by the appearance of a pair orsal melanophores bilateral to the midbrain. nd and third pairs appear in sequence immedi- posterior to the original ones. Shortly after appearance of the third pair, a melanophore be seen above the anterior branchial region. nentation spreads anteriorly and posteriorly the dorsal pairs along the neural tube (fig. it spreads posteriorly and ventrally from the al pair along the line between the yolk and the tes, as far as the anterior limit of opaque or fferentiated tissue. As transparency pro- ses, the posterior distribution of melanophores tended correspondingly.

ıe end-point of stage 15 is reached with the earance of the gill slits.

Stage 16: Gill-cleft (fig. 24) days 15-17

Size: 6 to 7.5 millimeters.

Neural tissue: Divisions of central nervous system recognized through ectoderm.

Gut: Cylindrical yolk mass. Increases in length and becomes more slender. No ventral flexion. Anterior portion greenish. Postanal gut present. Anus forms at persistent blastopore.

Mouth: Semicircular slit. Enlarges to hooded mouth. Oral cirri make first appearance.

Somites: No longer useful as staging criterion.

Locomotion: Larval swimming pattern. Very adept at end of stage.

Nostril: Migrates from anteroventral to anterodorsal extremity.

Transparency: Practically entire prolarva becomes transparent except gut.

Circulatory system: Ventricular wall has thickened. Heart beat, 150 per minute. Flow of blood readily visible.

Liver: Size increases. Distinctly separated from gut.

Pigmentation: Melanophores extend along dorsum and sides of embryo from anterior to posterior. Bilateral eyespots appear at end of stage.

Gill clefts: First appearance. They become functional.

Respiratory rate: 120 per minute.

Fins: Caudal and anal fins appear.

The mouth continues to migrate anteriorly and

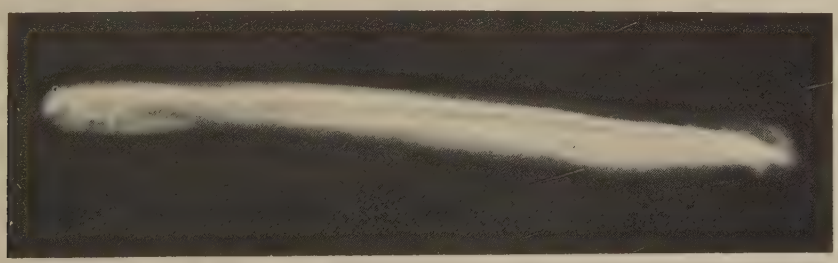


FIGURE 23.—Dorsal view of stage 15 showing condition of pigmentation over the head region.

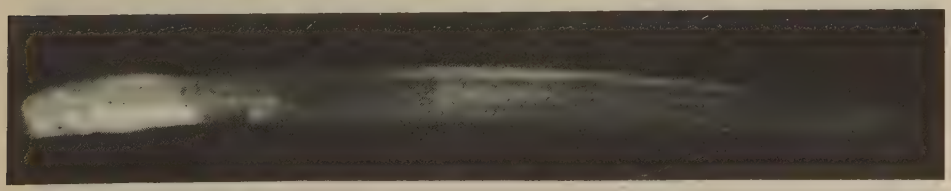


FIGURE 24.—Lateral view of stage 16, gill cleft, showing gill clefts, somites, pericardial area, and pigmentation over yolk mass.

the nostril dorsally. The anterior lip of the slit-like mouth of stage 15 moves anteriorly at the midline only, producing a semicircular mouth. Migration of the anterior lip and nostril continues until the mouth lies at the anteroventral extremity and the nostril is at the anterodorsal extremity. In later phases of the stage the mouth enlarges and is hooded by the anterior lip. Oral cirri begin to form posterior to the anterior and posterior lips.

Movement during this stage is by a rapid undulation of the trunk and tail. All ventral flexion of the tail region has disappeared during the transition from stage 15 to 16.

The anterior boundary of the auricle lies over the center of the ventricle. The flow of blood through the heart and other parts of the prolarva is seen readily.

In the latter period of this stage the bilateral pigmented retina (eyespot) appears anterior and dorsal to the velum. Melanophores which extend down the length of the neural tube and along the yolk-filled gut begin to migrate ventrally from both levels. Melanophores migrate along the myosepta from the neural-tube level and ventrally from the yolk-somite line. The anterior lip and head region also are well pigmented.

The appearance of gill clefts marks the initiat of stage 16. They appear and start function in order from the first to the seventh. Functi ing of the branchial apparatus can be determi by two means: beating of the velum (betwe the oral cavity and the first visceral cleft) as forces water through the pharynx; or, contracti of the gill clefts. The respiratory rate (vel beat) is 120 per minute.

The end-point of stage 16 is reached when t prolarvae acquire bilateral eyespots and are ab to burrow.

Stage 17: Burrowing (fig. 25) days 17-33

Size: 7.5 to 9.0 millimeters.

Gut: Cylindrical and yolk-filled. Postanal g absorbed. Esophagus visible on left side. Cloa transparent. Peristaltic movements in hind g Lumen of gut opens at end of stage.

Mouth: Oval with dorsal and ventral li Opens anteriorly. Oral cirri.

Locomotion: Swimming movements same as stage 16. Prolarvae burrow.

Nostril: On dorsal aspect, its ultimate positio

Transparency: Complete except for yolk-fill gut.

FIGURE 25.—Lateral views of stage 17, burrowing, showing the condition of the mouth and the lips.

rculatory system: Heart walls thicken. t beat more than 200 per minute. Flow of visually traceable throughout most of rva.

ver: Further increases in size. First indica- of gall bladder on right side.

gmentation: Melanophores extensively dis- ted. Aggregation over pronephric region audal end of notochord and neural tube.

spiratory rate: 200 per minute.

onephros: First detectable externally.

e posterior region of the gut becomes trans- t as the cloaca is formed with the opening e pronephric ducts into the gut (histological vations). A yolk pellet forms in the gut ante- o the cloaca as the result of peristaltic move- s of the gut. These movements move the toward the anus and then back to the ning yolk mass.

e lips take on the characteristic larval form. mouth opens directly anteriorly, so that the ior lip has now become the dorsal lip and the rior lip the ventral.

ge 17 begins when the prolarvae burrow into ottom mud. Burrowing is the result of the n of both the tail and the head regions. As ead moves from side to side to create space n the mud, the lashing of the tail drives the va into the mud. Prolarvae of this stage l in aquaria or beakers first swim near the e of the water and then suddenly plunge ward with rapid swimming movements until reach the bottom, when they immediately to burrow. Prolarvae of stage 16 merely lown to and lie on the bottom. Swimming ments do not differ from those of stage 16. e liver is extended farther and the presence e gall bladder in the later period is marked bile-green color.

e eyespot or retina is very prominent anterior lorsal to the velum. Melanophores have l completely around the gut region to the al surface. They have migrated down the l surface of the velum and the gill bars, have completely outlined the branchial t. The dorsal lip is covered completely melanophores.

ronephros dorsal and posterior to either side of ricardial cavity is visible externally because presence of much blood. This stage is the n which the pronephros is visible externally.

The end-point of stage 17 is reached when the lumen of the yolk-filled gut is opened.

Stage 18: Larva (fig. 26) days 33-40

Size: 9 millimeters and longer.

Gut: Lumen completely opened. Yolk extruded from gut. Gut tissue becomes transparent.

Respiratory rate: 200 per minute.

FIGURE 26.—Various views of several stage 18 larvae on a black background. Shown are: condition of the mouth and lips, eyespot, pigment outlining branchial region, liver immediately posterior to the heart, somites, fins, and the gut from the liver to the cloaca. The granular appearance along the gut is caused by pigmentation.

The transition from the prolarval to the larval condition (all systems differentiated save genital) is marked by the differentiation of the formerly yolk-filled gut into its definitive form. This change is seen outwardly as the gut becomes transparent at the "stomach" region behind the liver. Transparency progresses posteriorly until the entire gut has differentiated and the digestive system is open from the mouth to the anus. After the pellet described in stage 17 is passed, yolk remaining within the lumen of the gut is extruded constantly from the anus.

Stage 18 larvae are between 9 and 10 millimeters long at the time the gut becomes fully differentiated. They belong to the first larval stage which would be equivalent to age-group 0, 11 to 21 millimeters long, of Applegate (1950).

REVIEW OF STAGES AND COMPARISON WITH EARLIER STUDIES

Staging of lampreys in most early literature lacked precision. Investigators, for example, Shipley 1885, Scott 1887, McClure 1893, Hatta 1914, 1915, who studied early embryology, gave little or no attention to the need for staging. Developmental sequence was described in conventional embryological terms.

The first serious attempt to show stages of lamprey development was by Damas (1944) in his excellent histological study of *L. fluviatilis*, concerned primarily with tracing development of the head. Each section (comparable to a stage) comprised a short account of external features and a thorough histological description. His staging series began with embryos possessing 3 somites (section 1). The next section included embryos with 10 somites. (Every somite listed by Damas was not visible in external view, but each was seen histologically.) On the basis of somite numbers Damas was able to describe several stages slightly different from each other which could be described also by staging based on external morphological features. Some of his sections, based usually on intervals of 10 somites, have been translated into stages of the present study for comparison in table 1.

Stages described in the present work have been based primarily on changes of external features. Histological observations have been used sparingly and merely for clarification. Damas, who began his study after development had started, omitted the first 9 stages of the present work. Because his criteria were histological, his stages are overlapped by stages in the present study (table 1).

TABLE 1.—*Equating of "sections" of Damas (1944) study with "stages" of the present research*

Damas (section)	I	II	III	IV	V
Piavis (stage)	10	11	12	12	13
Damas (section)	VI	VII	VIII	IX	X
Piavis (stage)	13	13	14	14	14
Damas (section)	XI	XII	XIII	XIV	XV
Piavis (stage)	15	15	15	16	18

Staging, to be of practical value, should be simply, easily, and promptly recognized by investigators in both living and preserved materials. By far the easiest method of designating stages is one based on morphological characteristics since the resulting demarcations are relatively sharp and distinct and the diagnostic features are visible without elaborate histological preparat They are natural divisions in a developme sequence. Particular effort was made in study to avoid dependence on measurements counts. Ideally, observation alone should su for recognition of stages.

Applegate (1950) divided the larval forms *P. marinus* on the basis of length and weight i age groups, beginning with age-group 0. stated that the larvae ". . . upon leaving nest have completed their early developme stages and are perfectly formed but diminut ammocoetes." These ammocoetes are in rea stage 18 larvae, for specimens from Applega collections match specimens from my collecti in their external as well as histologic characterist

Stages, as designated here, can be determi by the naked eye or with a hand lens. Separat of stages 12 and 13 might conceivably cause so difficulty since transition between them is ba on muscular activity. It is easy, however, recognize preserved specimens, for the head reg of embryos of stage 13 is displaced to either s of midline whereas heads of stage 12 embryos medial.

Cleavage in the sea lamprey has several intere ing characteristics, the first of which is the appe ance of relatively high prominences lateral to a above the first cleavage furrow. Similar pro nences occur during second cleavage (stage 3).

McClure (1893) noted the unusual cleava pattern of *P. marinus*, especially in the third a fourth cleavages. He described the third cleava as meridional and considered this to be the s type of third cleavage. Actually, both meridio and equatorial types of third cleavage occur, b their relative abundance fluctuated widely ir spective of temperature.

Teleostei, Gymnophiona, Gallus, and oth forms exhibit a meridional third cleavage, whic however, is accomplished in meroblastic divisio In the sea lamprey the meridional third cleava is holoblastic.

The cleavage of stage 4 embryos determines t type of cleavage for stage 5, since an equator stage 4 is followed by meridional stage 5 a vice versa.

Cleavage of stage 6 embryos is indeterminat and is accompanied by a lag in the cleavage the vegetal cells. After stage 6, demarcation b tween the animal and vegetal cells is sharp un

egetal cells are covered by the epibolizing
al cells.

lastopore formation, as was observed by
ey (1885), involves behavior of animal cells
egetal cells similar to that in amphibians and
sts. When invagination of animal cells
s the blastopore, the margin of animal cells
anged latitudinally around the embryo from
lastopore. As the animal cells continue to
lize, the vegetal cells are covered by animal
from the anterior and dorsolateral portions
e animal hemisphere and subsequently from
posterolateral and ventral positions in the
n described for stage 9. Thus, migration of
al cells over vegetal cells in the lamprey
hes closely the epiboly of animal cells in
ibians. The dorsal lip does not curve as
, however, in the sea lamprey as in amphib-
. If the blastopore is considered to be de-
by the line of epibolizing animal cells, as it is
eosts, the blastopore of the sea lamprey is at
oval. This shape results from alignment of
al cells lateral to the midsagittal plane. A
ar blastopore is formed when animal cells
e ventralmost point of the oval-shaped
opore cover the oval yolk plug in a ventral-
rsal direction along the mid-sagittal plane.
e circular blastopore of the sea lamprey
rently migrates toward the center of the
al hemisphere whereas the teleost blastopore
tes toward the vegetative pole as the embryo
hens. The amphibian blastopore eventually
es and passes beyond the vegetative pole at a
equivalent to stage 13 of the lamprey.
use teleost embryos do not extend ventrally
the yolk mass the blastopore does not reach
egetative pole.

e apparent migration of the lamprey blasto-
might possibly be attributed to the decrease
e volume of the blastocoel during stages 9
10. An increase in embryo volume which
place in stage 8 is apparently due primarily
e increased volume of the blastocoel. During
9, after involution is completed, the volume
e embryo decreases as the result of a decrease
e volume of the archenteron. The archen-
, in reality, is the original blastocoel since
lastocoel is not obliterated during gastrula-
(histological observations) as it is in amphib-
In this feature of gastrulation the lamprey
ly resembles teleosts. Since decrease in the size of the archenteron shortens the embryo and since growth of the neural tube in stages 12 and 13 moves the blastopore along the mid-sagittal plane, the blastopore appears to migrate.

The present study also corroborates Shipley's (1885) observation that the open blastopore persists, eventually becoming the anus. Histological sections permit tracing the archenteron to an open blastopore through stage 15. During stage 16, the diameter of the blastopore widens and the lips thicken to form the anus. The archenteron can be traced to the anus in stage 16 as it was traced to the blastopore in stage 15.

Shipley recognized that the early neural tube did not possess a neurocoel and called it a neural rod, which term has been retained here. Selys-Longchamp (1910) described neural-tube formation in lampreys as intermediate between the keel method of teleosts and neural-fold method of other vertebrates.

Among the morphological features prominent during development were the gut, liver, gall bladder, hemoglobin and vascularization, and pigment. The gut opens from the oral cavity to the anus at stage 18. The stomodaeum opens in stage 16, the esophagus in stage 17, the cloaca in stage 17, and finally the portion between esophagus and cloaca at stage 18.

The formation of the liver in late stage 14 is indicated by the greenish cast of the anteriormost portion of the gut. Vascularization of the liver occurs during stage 15. The size of the liver continues to increase through stages 15 and 16. A gall bladder forms in stage 17; it is recognizable externally by its accumulation of blue-green bile.

Additional changes in stage 15 embryos include the appearance of hemoglobin within the blood channels which had formed in the yolk-filled gut. Blood cells appear first in the midventral channel and soon are in all three major channels. Blood formation and the bulk of vascularization take place in stages 15 and 16. Vascularization is extended in stage 17, when practically all major vessels can be traced by following red corpuscles within the transparent tissues.

Pigmentation of the embryo begins as two melanophores bilateral to the midbrain. Successive pairs of melanophores appear posterior to the initial one; next is the appearance of a melanophore above the branchial region. The number of melanophores increases throughout stage 15 when

they loosely cover the dorsum and form a line posteriorly from the branchial region between yolk and somites. In stage 16 melanophores extend along the dorsum and sides of the embryo. Bilateral eyespots appear at the end of stage 16. The melanophores become more extensively distributed in stage 17. Aggregations appear over the pronephric region and the caudal end of the notochord and neural tube. The anterior lip, and the head region and the gut become profusely covered with pigmentation. The pattern of pigmentation changes little in stage 18 except that the pigmented area is extended.

Activity in the embryo included locomotory movements, heart beat, and velum beat. First evidence of activity is the movement of the head region, produced by muscles of somites of stage 13 embryos. Greater muscular movements take place during later stages, including the movements that lead to: hatching; poor swimming movements of stage 14; somewhat better swimming in stage 15; the greatly increased deftness of swimming in stage 16; and movements which accomplish burrowing at stage 17.

Changes in the heart region in stage 14 embryos can be seen through the ectoderm over the pericardial cavity as it becomes transparent. The heart begins to beat at a rate of 40 times per minute, increasing to 100 per minute in stage 15, 150 per minute in stage 16, and more than 200 per minute in stage 17.

The respiratory system becomes functional at stage 16 when the initial respiratory rate (velum beat) is 120 per minute. This rate increases to 200 per minute in stage 17 and is maintained at this rate into stage 18.

In general, lamprey development resembles amphibian development in cleavage (stages 1–8). Lamprey gastrulation resembles amphibian gastrulation in some respects and teleostean in others. Epiboly is more like that of amphibians than of teleosteans. Invagination and involution of chorda-mesoderm seem similar to that in amphibians. Formation of the lamprey archenteron produces a situation comparable to that of a teleost. In both, the developing embryo is located on a large yolk mass but separated from the yolk by an archenteron which was the original blastocoel. A major difference between the two is that lamprey yolk is divided whereas teleostean yolk remains undivided. Another similarity to teleostean development is the formation of a sc neural rod which develops a lumen only af neurulation has been completed.

DEVELOPMENT AT DIFFERENT CONSTANT TEMPERATURES

The primary objective of the experimen rearing of sea lampreys at a series of consta temperatures was to determine the temperatu levels at which the eggs were capable of developi into normal, viable larvae. As part of the wo detailed records were kept on: the relation betwe temperature and progression of developmer mortality rate during development; occurrence a nature of developmental abnormalities. Inf mation of this type may help explain the failur noted both in the United States and in Canad of the sea lamprey to utilize certain apparent suitable spawning streams as extensively as oth apparently similar waters.

MATERIALS AND METHODS

The experiments were conducted at temper tures and with the aid of equipment indicated table 2. Throughout this work, control lots 65° F. were maintained as an index to develo ment. Thus, any one control lot could ser effectively as an indicator to several other exper ments. Eggs from 2 to 4 females were mixed wit sperm from 4 to 8 males. These eggs were the apportioned into containers in the numbers (b actual count) indicated in table 3.

TABLE 2.—*Equipment used in experiments on developme at constant temperatures*

Temperature (° F.)	Temperature-control equipment	Temperature (° F.)	Temperature-control equipment
45	Refrigeration.	65	Heat, refrigeratio (Bronwil circulator)
50	Refrigeration.	70	Heat.
52.5	Refrigeration.	75	Circulator.
55	Refrigeration.	77.5	Circulator.
60	Heat and refrigeration.	80	Circulator.

The sampling schedule differed somewhat amon the experiments (as may be seen from later table that give details for individual samples) but th differences of schedule and the unavoidable occa sional interruptions of timing were not sufficien to impair comparisons between series or to hampe the description of the progress of development In the main, the earlier samples were taken a 1- or 2-hour intervals; the time between sample

LE 3.—*Specimens and number of eggs used in experiments on development at constant temperatures*

group of lampreys provided the eggs for experiments conducted at 45°, 70°, 75°, and 80° and another group for those at 52.5°, 60°, and 77.5°. gs from the lots that supplied materials for experiments at 50° and 65° e reared at the one temperature only]

Water temperature (° F.)	Number of females	Number of males	Number of eggs
	4	8	10,000
	2	4	10,000
	2	4	15,000
	4	8	25,600
	2	4	9,000
	2	4	33,000
	4	8	18,000
	4	8	13,500
	2	4	9,000
	4	8	5,000

increased to 4 hours at about 32 hours; later nples (that is, after 2–3 days) were obtained ut 12 hours apart; and, finally in the longer eriments sampling was daily. Sampling after ge 14 appeared was biased because the pro-rae had to be pursued as they became older; s the samples were non-representative in com-ison with the random samples for all earlier ges. In the main the bias led to over-repre-tation of live specimens.

everal criteria were used to separate live and d embryos. The most obvious indication of th was disintegration of the embryo—a sepa-on of cells and subsequent filling of the intra-mbrane volume with loosely arranged cells, hin an intact membrane. Furthermore, the ilization membrane became translucent with loudy cast in contrast to the transparency of iving membrane. This difference was most arent in later development, stages 7–14; the lier stages (1–6) became vacuolated as they lerwent changes after death. Eventually, the mbranes of eggs in these earlier stages also be-ne cloudy. Another indicator, particularly in y development, was a change of color from the amy white of normal eggs to a brownish tan, ompanied by a fuzzy appearance of the surface. ing embryos of stages 9–11 possessed appreci-y widened blastopores, which in some extended oss the entire diameter of the embryo.

The basic data on the several temperature eriments are given in the records of number of d embryos and number of living embryos in ious stages of development. In conjunction h these records, information was recorded tive to the elapsed time and temperature and remarks were noted concerning the general composition of each sample.

The percentage of dead embryos was computed for each sample. Until stage 14 appeared this percentage provided an estimate of mortality up to the time of sampling since all embryos regardless of time of death were included in the computations. This procedure was possible since all eggs used in any one experiment were recoverable throughout the experiment. Although disintegration of dead embryos did occur, the embryonic membrane remained intact to the end of the experiment. The information on dead embryos per sample does not include data on time of death, since stages could not be determined for embryos that were decomposing, but it does provide good information on the progression of mortality with development.

Because, as has been stated, the quantitative sampling of lots of eggs become biased after the appearance of stage 14 (hatching) and later stages, none of the tables that carry details on individual samples goes beyond the last sample that contained stage 13 embryos (the bias starts, of course, with the first sample containing stage 14 specimens, but full records for stage 13 appear to be desirable). In the experiments in which stage 14 was not reached, the tabulation ends with the last sample that contained live embryos. Similarly, the tables showing mortality by 1-day intervals end with the records for the first day on which stage 14 appeared, or, if that stage was not reached, with the last day on which samples contained live embryos. Terminal samples are included for those experiments in which some embryos survived the full term of the experiment or in which there was cause to suspect that a few live embryos might still be present. These terminal samples which contained all eggs remaining at the end of the experiment are considered, for practical purposes to be unbiased. They were affected to some degree by the earlier removal (after the start of stage 14) of samples in which living embryos were taken out of proportion to their true abundance, but the numbers were so much greater in the terminal sample than in these earlier biased samples as to minimize the distorting effect.

Records from biased samples were of course useful for showing the time of first appearance and duration of the later stages.

ACCOUNT OF INDIVIDUAL EXPERIMENTS

Development at 45° F.

The sea lamprey eggs could not make even a good start toward development in a constant temperature of 45° F. The first two cleavages (stages 1 and 2) proceeded very slowly and all eggs were dead before the third cleavage was completed (tables 4 and 5; fig. 27).

TABLE 4.—*Living and dead lamprey embryos per sample and their stage of development at 45° F. (7.2° C.)*

Sample	Number of hours	Number dead	Stage of living embryos			Sample	Number of hours	Number dead	Stage of living embryos		
			1	2	3				1	2	3
1	1	67	213	----	----	14	27	91	----	23	----
2	3	15	105	----	----	15	31	105	----	57	----
3	5	10	94	----	----	16	35	120	----	23	----
4	7	13	125	----	----	17	39	123	----	32	----
5	9	16	169	----	----	18	43	85	----	16	----
6	11	17	120	----	----	19	47	177	----	22	2
7	13	11	113	----	----	20	51	207	----	8	4
8	15	73	92	----	----	21	58	175	----	----	10
9	17	38	233	----	----	22	71	252	----	----	14
10	19	82	84	33	----	23	82	322	----	----	3
11	21	45	76	109	----	24	95	363	----	----	2
12	23	45	----	101	----	25	103	341	----	----	2
13	25	62	----	63	----						

TABLE 5.—*Mortalities of sea lamprey eggs reared at a constant temperature of 45° F. (7.2° C.)*

Time		Stage span	Number of samples	Number of embryos			Percentage dead
Hours	Days			Alive	Dead	Total	
0–24	1	0–2	12	1,667	432	2,099	21
25–48	2	2–3	7	238	763	1,001	76
49–72	3	2–3	3	36	634	670	95
73–96	4	3	2	5	685	690	99
97–120	5	3	2	2	579	581	[1] 100

[1] More than 99.5; actually all embryos (238 specimens) were dead in the sample taken at 116 hours.

Stage 1 lasted about 20 hours; stage 2 commenced at the 19th hour and lasted beyond the 47th hour, when a highly defective stage 3 appeared (tables 4 and 6; fig. 28). Many 3-cell embryos were found along with a few abortive 4-cell embryos, in which the cleavage furrow seemed to undergo regression.

Throughout the samples, beginning with the sixth at 11 hours, the eggs began to vacuolate and to fragment yolk into the perivitelline space, in short, to die and disintegrate. Beginning with the 19th sample, at 47 hours nearly 90 percent

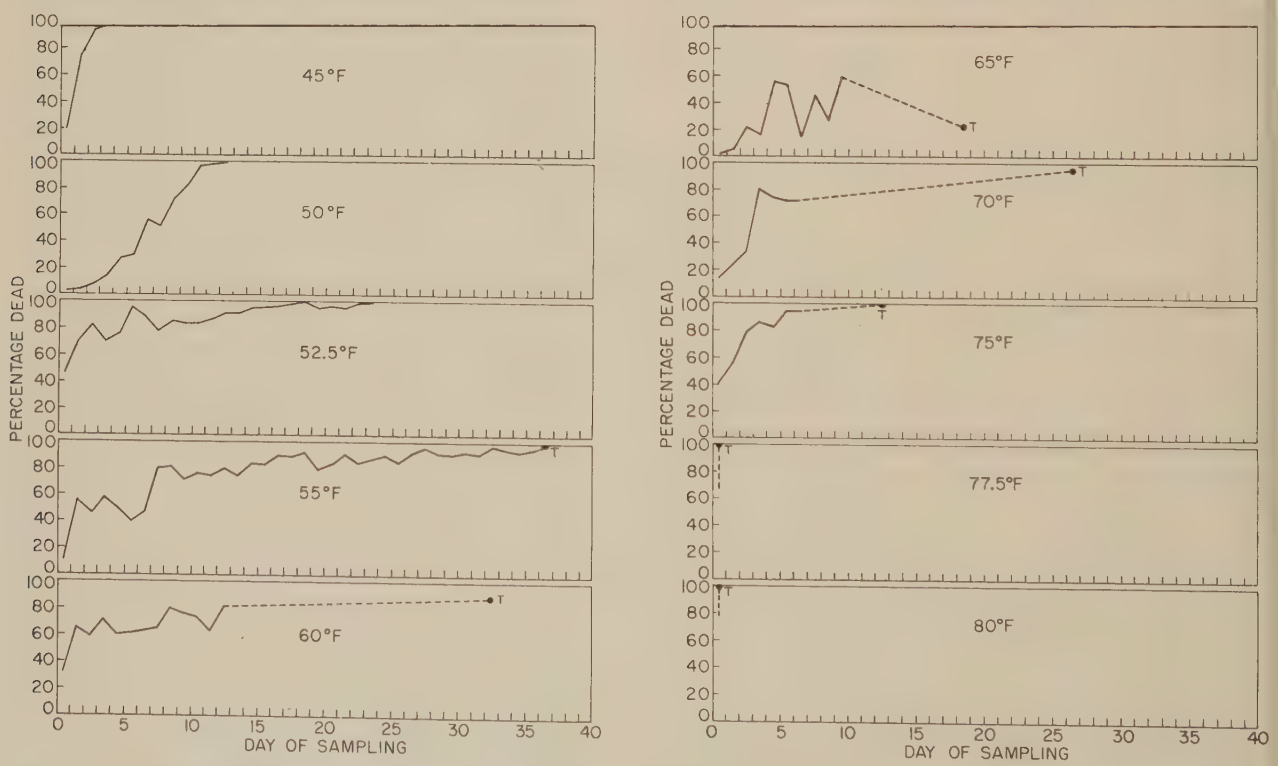


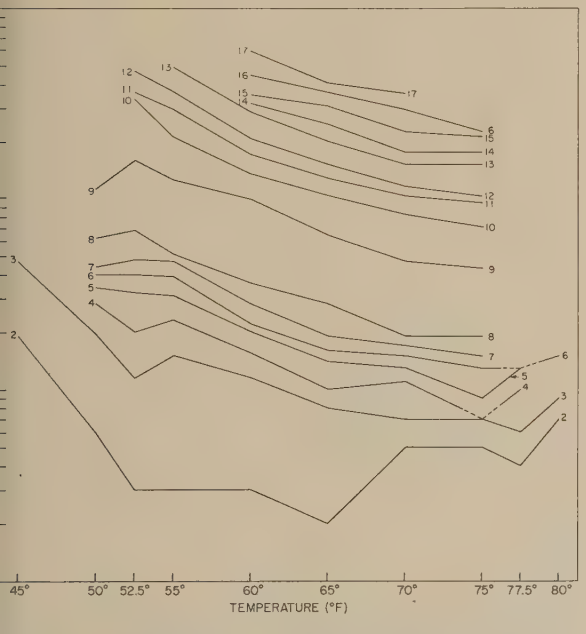
FIGURE 27.—Percentage of dead embryos in daily samples from sea lamprey eggs reared at 10 different constant temperature levels. The day-by-day records end when the percentage of dead in the samples reached and remained at 100, or with the onset of hatching. A broken line connects the last recorded daily sample with the terminal sample (T). At 77.5° and 80° live embryos occurred in the earlier samples of the first day but none in the later ones.

BLE 6.—*Hours at which the first and last specimens of the various stages appeared in samples during each experiment and the duration of stages (in parentheses)*

Stages	Constant temperature (F.) at which embryos were reared									
	45°	50°	52.5°	55°	60°	65°	70°	75°	77.5°	80°
	1–21	1–16	1–10	1–13	1–6	1–2	1–3	1–3	1–4	1–7
	(21)	(16)	(10)	(13)	(6)	(2)	(3)	(3)	(4)	(7)
	19–51	6–25	3–22	3–23	3–10	2–8	5–7	5	4–6	5–9
	(33)	(20)	(20)	(21)	(8)	(7)	(3)	(2)	(3)	(5)
	47–103	19–34	13–28	15–31	13–16	8–11	7–11	7	6–10	9–11
	(57)	(16)	(16)	(17)	(4)	(4)	(5)	(2)	(5)	(3)
	----------	28–42	20–36	23–35	16–18	10–15	11–13	7–11	10	[3]
	----------	(15)	(17)	(13)	(3)	(6)	(3)	(5)	(4)	----------
	----------	34–48	32–44	31–43	20–22	13–15	13–15	9–13	[2] 13	[3]
	----------	(15)	(13)	(13)	(3)	(3)	(3)	(5)	----------	----------
	----------	40–58	40–48	39–47	22–28	16–19	15–17	13–15	[2] 13	[2] 15
	----------	(19)	(9)	(9)	(7)	(4)	(3)	(3)	----------	----------
	----------	44–78	48–64	47–103	28–34	19–24	17–21	15–17	----------	----------
	----------	(35)	(17)	(57)	(5)	(6)	(5)	(3)	----------	----------
	----------	62–126	68–136	51–151	36–89	28–64	19–51	19–43	----------	----------
	----------	(65)	(69)	(101)	(54)	(37)	(33)	(25)	----------	----------
	----------	114–270	160–338	127–271	100–148	64–104	47–82	43–82	----------	----------
	----------	(167)	(179)	(145)	(49)	(41)	(36)	(40)	----------	----------
	----------	----------	338–362	211–295	136–172	104–128	82–103	71–103	----------	----------
	----------	----------	(25)	(185)	(37)	(25)	(22)	(33)	----------	----------
	----------	----------	362–470	295–405	172–220	128–152	103–127	95–116	----------	----------
	----------	----------	(109)	(111)	(49)	(25)	(25)	(22)	----------	----------
	----------	----------	470–570	369–525	208–283	152–200	116–151	103–139	----------	----------
	----------	----------	(101)	(157)	(76)	(49)	(36)	(37)	----------	----------
	----------	----------	----------	[1] 490–874	283–409	200–296	151–235	151–175	----------	----------
	----------	----------	----------	[4] (--------)	(127)	(97)	(85)	(25)	----------	----------
	----------	----------	----------	----------	317–409	[4] 248–------	175–247	175–211	----------	----------
	----------	----------	----------	----------	(93)	[4] (--------)	(73)	(37)	----------	----------
	----------	----------	----------	----------	352–469	[4] 308–------	225–307	211–225	----------	----------
	----------	----------	----------	----------	(118)	[4] (--------)	(83)	(15)	----------	----------
	----------	----------	----------	----------	446–569	[4] 363–------	295–343	[1] 225–307	----------	----------
	----------	----------	----------	----------	(124)	[4] (--------)	(49)	[4] (--------)	----------	----------
	----------	----------	----------	----------	[1] 594–782	[1] 405–437	[1] 357–633	----------	----------	----------
	----------	----------	----------	----------	[4] (--------)	[4] (--------)	[4] (--------)	----------	----------	----------

[1] Termination of experiment.
[2] 100-percent mortality subsequent to this hour.
[3] No embryo of this stage found in any sample.

[4] These blanks cannot be filled properly for these stages were sampled either in or immediately preceding the terminal sample.



GURE 28.—Number of hours required by sea lamprey embryos to reach different developmental stages when reared at various constant temperatures.

of the eggs were dead. All eggs obviously were dead in samples taken after the 5th day (table 5). Eggs marked by lesions as described by Damas (1948) did not undergo first cleavage.

The highest stage completed in this experiment was stage 2, since stage 3 was not consummated.

Development at 50° F.

The rate of development was decidedly faster at 50° F. than at 45° F. (table 6; fig. 28) and development proceeded farther. Stage 9 was reached but few embryos survived to the end of the stage (tables 7 and 8; fig. 27). A single sample contained eggs that seemed to be intermediate between stages 9 and 10. No embryos were alive after the 270 hours (sample 65, table 7; table 6, also fig. 27).

The duration of each of the first 6 stages fell within the limited range of 15–20 hours (table 6). Stage 7, however, lasted 35 hours, and stage 8 approximately 65 hours, the longest save for the last stage reached (stage 9). The greatest period overlap of stages occurred between stages 7 and 8

TABLE 7.—*Living and dead embryos per sample and their stage of development at 50° F. (10° C.)*

Sample	Hours	Number dead	Stage of living embryos					
			1	2	3	4	5	6
1	½		93					
2	1		140					
3	2		215					
4	3		184					
5	4		133					
6	6	1	200	20				
7	7	1	157	43				
8	8		132	34				
9	10	1	108	30				
10	11		129	18				
11	12		132	27				
12	13		112	68				
13	14		31	84				
14	15		42	139				
15	16	1	14	111				
16	17	6		139				
17	18	6		151				
18	19	8		283	2			
19	20	11		304	62			
20	21	12		271	127			
21	22	8		141	213			
22	23	10		72	210			
23	24	6		47	262			
24	25	7		13	95			
25	26	5			117			
26	27	4			72			
27	28				48	12		
28	29				50	48		
29	30	18			137	190		
30	32	23			90	344		
31	34	19			26	453	23	
32	36					43	60	
33	38	3				24	80	

Sample	Hours	Number dead	Stage of living embryos					
			4	5	6	7	8	
34	40	3	7	60	5			
35	42	3	5	19	89			
36	44	17		69	232	8		
37	46	14		49	187	5		
38	48	10		8	83	30		
39	50	13			108	107		
40	54	25			41	163		
41	58	11			17	132		
42	62	15				87	183	
43	66	14				13	125	
44	70	13				11	271	
45	74	35				5	335	
46	78	71				2	236	
47	90	32					238	
48	102	87					119	
49	114	42					209	
50	126	32					77	
51	137	93						
52	146	43						
53	150	123						
54	163	119						
55	171	118						
56	174	207						
57	182	169						
58	187	232						
59	198	337						
60	211	315						
61	222	283						
62	234	254						
63	246	375						
64	260	435						
65	270	634						

TABLE 8.—*Mortalities of sea lamprey eggs reared at a constant temperature of 50° F. (10° C.)*

Time		Stage span	Number of samples	Number of embryos			Percentage dead
Hours	Days			Alive	Dead	Total	
0–24	1	0–3	23	4,680	71	4,751	2
25–48	2	3–7	15	2,781	126	2,907	4
49–72	3	6–8	6	1,258	91	1,349	7
73–96	4	7–8	3	816	138	954	14
97–120	5	8–9	2	335	129	464	28
121–144	6	8–9	2	306	125	431	29
145–168	7	9	3	223	285	508	56
169–192	8	9	4	684	726	1,410	52
193–216	9	9	2	229	652	881	74
217–240	10	9	2	107	537	644	83
241–264	11	9	2	12	810	822	99
265–288	12	9	2	6	1,222	1,228	99.5

(20 hours) and the next longest between stages 8 and 9 (12 hours). Mortality was light the first 3 days (2 to 7 percent dead; table 8) and was moderate (14 to 29 percent dead) the next 3 days. The death rate increased considerably shortly after the embryos entered stage 9 (gastrula). Samples on the seventh day contained 56 percent of dead eggs. The percentage had reached 99 and 99.5 percent on the 11th and 12th days, respectively, and all eggs were dead on the 13th day.

Development at 52.5° F.

Again, an increase of temperature (this time only 2.5° above that of the experiment described in the preceding section) permitted development at a faster rate and through a greater number o stages (table 6; fig. 28). Heavy mortality starte early, however, the death rate increased as deve opment progressed (tables 9 and 10; fig. 27), an no embryos survived beyond stage 12 (head stage

The early stages (1 to 6) of this experiment pro ceeded slightly more rapidly than the correspond ing stages of the 50° F. experiment. The perio of overlap of the earlier stages were nearly equa to those of the 50° F. test, although the length o time for each stage was less at 50° F. than at 52.5 (tables 6, 7, and 9). Stage 9 was prolonged a 52.5° F. over a period of approximately 9 days During this time the number of deaths increased The relatively few embryos that developed beyon stage 9 progressed through a short stage 10 and much longer stage 11 (about 4 days). Stage 1 was reached by approximately 40 embryos, all o which died. The most frequent symptom of deat was disintegration of the anterior region of th embryos.

Development at 55° F.

Development at 55° F. proceeded as far a stage 13 (prehatching). Indeed, the termina sample included 11 live embryos in this stag (along with some 16,000 dead embryos) but all o them were so defective as to make early deat almost certain; they were accordingly classed a

TABLE 9.—*Living and dead embryos per sample and their stage of development at 52.5° F. (11.4° C.)*

Sample	Hours	Number dead	Stage of living embryos								Sample	Hours	Number dead	Stage of living embryos				
			1	2	3	4	5	6	7	8				8	9	10	11	12
	1	2	76								28	136	142	6				
	3	11	29	2							29	148	37					
	5	15	23	4							30	160	33		10			
	6	7	76	3							31	172	58		20			
	8	8	36	2							32	814	57		12			
	10	21	31	1							33	196	76		13			
	13	31		15	1						34	281	44		12			
	14	47		23	2						35	230	70		10			
	16	38		9	1						36	242	65		12			
	18	74		12	3						37	254	86		17			
	20	27		2	10	1					38	269	40		6			
	22	37		2	12	3					39	293	75		9			
	24	48			7	3					40	302	78		7			
	26	33			2	10					41	327	53		5			
	28	39			2	16					42	338	64		4	1		
	32	29				13	12				43	349	96			4		
	36	44				2	11				44	362	85			2	1	
	40	49					2	10			45	375	96				5	
	44	66					2	15			46	386	79				2	
	48	29						4	21		47	419	117				2	
	54	51							18		48	447	44					
	64	65							5	2	49	470	83				1	3
	76	21								9	50	494	98					3
	89	46								20	51	518	68					3
	100	32								13	52	543	79					1
	112	72								21	53	570	92					1
	124	50								5								

BLE 10.—*Mortalities of sea lamprey eggs reared at a constant temperature of 52.5° F. (11.4° C.)*

Time		Stage span	Number of samples	Number of embryos			Percentage dead
Hours	Days			Alive	Dead	Total	
	1	0–4	13	389	366	755	48
	2	3–7	7	122	289	411	70
	3	7–8	2	25	116	141	82
	4	8	2	29	67	96	70
0	5	8	2	34	104	138	75
44	6	8	2	11	192	203	95
68	7	9	2	10	70	80	88
92	8	9	2	32	115	147	78
16	9	9	1	13	76	89	85
40	10	9	2	22	114	136	84
64	11	9	2	29	151	180	84
88	12	9	1	6	40	46	87
12	13	9	2	16	153	169	91
36	14	9	1	5	53	58	91
60	15	9–10	2	9	160	169	95
84	16	10–11	2	8	181	189	96
08	17	11	1	2	70	72	97
32	18	11	1	2	117	119	98
56	19		1		44	44	100
80	20	11	1	4	83	87	95
04	21	12	1	3	98	101	97
28	22	12	1	3	68	71	96
52	23	12	1	1	79	80	99
76	24	12	1	1	92	93	99

ead" in the records for that sample (tables 11 1 12; fig. 27). The incidence of dead embryos the samples increased rather consistently as velopment proceeded and was high in the later ges.

Overlapping was prominent between later stages ce individual stages lasted from 4 to 16 days ble 6; fig. 28). Stage 13, the highest stage ched, had lasted 384 hours (16 days) when the eriment was finally terminated at 874 hours . 37 days). Stages 12 and 13 overlapped hours.

A most interesting feature of this experiment was the condition of embryos that finally reached and remained in stage 13. Although they were in stage 13, they were developing within the fertilization membrane (chorion). The spirally curled embryos developed transparent pericardia, pigment spots, and hemoglobin which had a muddy red to brown appearance in contrast to the normal bright pink to red. Some of these embryos finally were released when their membranes disintegrated. After release, however, the embryos did not straighten out or develop further but remained in this condition until death.

During this test several abnormalities were noted in the embryos: enlarged pericardia; straight tubular hearts; shorter but heavier bodies; enlarged gut region; a separation of yolk cells from the gut walls; and failure to straighten from the spirally curled position after removal of the chorion.

Development at 60° F.

The rate of development was much more rapid and the overlap of stages was less at 60° F. than at lower temperatures (tables 6, 13, and 14; fig. 28). The temperature of 60° F. was the lowest at which viable, burrowing prolarvae (stage 17) were produced. Mortality was generally less than on corresponding days in experiments at lower temperatures (fig. 27) and abnormalities were relatively few.

TABLE 11.—*Living and dead embryos per sample and their stage of development at 55° F. (12.8° C.)*

Sample	Hours	Number dead	Stage of living embryos								
			1	2	3	4	5	6	7	8	9
1	1		143								
2	3		80	8							
3	5		91	12							
4	7		27	53							
5	9		64	67							
6	11		32	114							
7	13		9	92							
8	15	22		91	19						
9	17	48		31	91						
10	19	14		56	65						
11	21	15		26	40						
12	23	47		5	34	48					
13	25	61			26	40					
14	27	83			16	35					
15	31	81			4	52	23				
16	35	81				9	75				
17	39	81					25	57			
18	43	62					11	16			
19	47	87						25	52		
20	51	70							36	39	
21	58	89							50	77	
22	71	118							50	75	
23	82	157							38	83	
24	95	151							40	81	
25	103	111							28	98	
26	116	133								135	
27	127	51								34	49
28	139	89								82	59
29	151	20								152	68
30	163	226									61

Sample	Hours	Number dead	Stage of living embryos				
			9	10	11	12	13
31	175	91	26				
32	187	141	34				
33	199	146	41				
34	211	133	27	3			
35	225	80	21	14			
36	235	49	5	16			
37	247	54	6	10			
38	260	61	3	19			
39	271	30	2	13			
40	283	47		12			
41	295	41		8	10		
42	307	83			14		
43	319	47			18		
44	331	37			11		
45	343	64			15		
46	357	71			12		
47	369	97			17	2	
48	381	86			12	9	
49	393	63			3	5	
50	405	56			2	4	
51	417	62				6	
52	432	48				9	
53	456	108				11	
54	465	73				19	
55	490	97				12	
56	501	89				7	
57	512	99				2	
58	525	148				3	
59	538	71					
60	549	92					

TABLE 12.—*Mortalities of sea lamprey eggs reared at a constant temperature of 55° F. (12.8° C.)*

[No sample on 24th day]

Time		Stage span	Number of samples	Number of embryos			Percentage dead
Hours	Days			Alive	Dead	Total	
0–24	1	0–4	12	1,298	146	1,444	10
25–48	2	4–7	7	466	536	1,002	54
49–72	3	7–8	3	327	268	595	45
73–96	4	7–8	2	242	308	550	56
97–120	5	7–8	2	261	244	505	48
121–144	6	8–9	2	224	140	364	38
145–168	7	8–9	2	291	246	537	46
169–192	8	9	2	60	232	292	79
193–216	9	9–10	2	71	279	350	80
217–240	10	9–10	2	56	129	185	70
241–264	11	9–10	2	38	115	153	75
265–288	12	9–10	2	27	77	104	74
289–312	13	10–11	2	32	124	156	79
313–336	14	11	2	29	84	113	74
337–360	15	11	2	27	135	162	83
361–384	16	11–12	2	40	183	223	82
385–408	17	11–12	2	14	119	133	89
409–432	18	12	2	15	110	125	88
433–456	19	12	1	11	108	119	91
457–480	20	12	1	19	73	92	79
481–504	21	12–13	2	37	186	223	83
505–528	22	12–13	2	26	247	273	90
529–552	23	13	2	30	163	193	84
577–600	25	13	1	15	113	128	88
601–624	26	13	1	18	93	111	84
625–648	27	13	1	12	125	137	91
649–672	28	13	1	5	104	109	95
673–696	29	13	1	12	126	138	91
697–720	30	13	1	9	86	95	91
721–744	31	13	1	10	108	118	92
745–768	32	13	1	6	61	67	91
769–792	33	13	1	4	109	113	96
793–816	34	13	1	6	103	109	94
817–840	35	13	1	8	108	116	93
841–864	36	13	1	8	128	136	94
865–888	37	13	1	3	105	108	97
875 [1]	37		1		16,250	16,250	100

[1] Terminal sample includes 11 live embryos mentioned in text as being incapable of survival.

Overlapping of stages was most limited wit the exception of stages 1 and 2 (overlap of samples) and the last 2 (stages 13 and 14) (overla of 7 samples). Stage 13 was longest (127 hou or about 5 days). Stage 8 lasted more than 5 hours and was also represented in 8 sample (tables 6 and 13). Stage 14 was attained withi 317 hours (about 13 days for first appearance

Percentages of dead embryos in samples ros from a low of 32 percent during early cleavag stages (1–6) to 88 percent in the terminal sample The rate of increase was highest in the earl stages actually during the first 2 days. Percentag hatch (embryos that survived through stage 14 though not accurately measurable from th biased samples, obviously was good.

The relatively few abnormalities during thi experiment (approximately 20 percent) took several forms, some of which were similar t those described earlier. Among the more common were enlargement of the pericardial area, and the straight tubular heart which, nevertheless, maintained a regular beat. Other specimens exhibited abnormal curvatures, balloon mouths, or cleft-lip. Abnormal curvatures of the trunk region produced embryos with "C," "J," "O," and "L" shapes. Balloon mouths were caused

TABLE 13.—*Living and dead embryos per sample and their stage of development at 60° F. (15.5° C.)*

Sample	Hours	Number dead	Stage of living embryos							
			1	2	3	4	5	6	7	8
	1		143							
	3		31	13						
	4		14	20						
	6	12	8	56						
	8	11		38						
	10	13		26						
	13	17			26					
	14	22			25					
	16	29			9	14				
	18	37				29				
	20	24					17			
	22	22					23	3		
	24	53						23		
	26	36						11		
	28	31						4	13	
	32	34							19	
	36	30								12
	40	28								10
	44	28								23
	48	31								26
	54	17								15
	64	26								15
	76	16								13
	89	31								5

Sample	Hours	Number dead	Stage of living embryos					
			9	10	11	12	13	14
25	100	20	15					
26	112	22	13					
27	124	26	14					
28	136	35	19	6				
29	148	23	5	7				
30	160	36		23				
31	172	29		11	7			
32	184	31			14			
33	196	55			5			
34	208	23			9	6		
35	220	37			2	12		
36	232	68				18		
37	244	40				20		
38	259	49				11		
39	283	45				9	16	
40	292	46					10	
41	317	51					20	4
42	328	36					4	5
43	339	35					8	12
44	352	5					1	6
45	365						5	3
46	376	3						
47	409	32					4	11

ABLE 14.—*Mortalities of sea lamprey eggs reared at a constant temperature of 60° F. (15.5° C.)*

[No sample on 17th day]

Time		Stage span	Number of samples	Number of embryos			Percentage dead
Hours	Days			Alive	Dead	Total	
24	1	0–6	13	518	240	758	32
–48	2	6–8	7	118	218	336	65
–72	3	8	2	30	43	73	59
–96	4	8	2	18	47	65	72
–120	5	9	2	28	42	70	60
1–144	6	9–10	2	39	61	100	61
5–168	7	9–10	2	35	59	94	63
9–192	8	10–11	2	32	60	92	65
3–216	9	11–12	2	20	78	98	80
7–240	10	11–12	2	32	105	137	77
1–264	11	12	2	31	89	120	74
5–288	12	12–13	1	25	45	70	64
9–312	13	13	1	10	46	56	82
3 336	14	13–14	2			120	
7–360	15	13–15	2			75	
1–384	16	13–15	2			31	
9–432	18	13–15	1			70	
3–456	19	15–16	1			22	
7–480	20	15–16	1			20	
1–504	21	16	1			16	
5–528	22	16	1			17	
9–552	23	16	1			42	
3–576	24	16	1			19	
7–600	25	17	1			18	
1–624	26	17	1			24	
5–648	27	17	1			35	
9–672	28	17	1			17	
3–696	29	17	1			12	
7–720	30	17	1			14	
1–744	31	17	1			8	
5–768	32	17	1			15	
9–792	33	17	1			7	
3 [1]	33	17	1	795	5,606	6,401	88

[1] Terminal sample.

rom a failure of the stomadaeum to open. An normous enlargement or ballooning of the haryngeal region resulted, especially in the nterior portion, which tended to compress the ranchial structures and cause them to become alformed and from all indication, non-functional. A midline cleft in the dorsal lip was designated a "cleft-lip" abnormality.

Development at 65° F.

The rearing of sea lamprey eggs under constant temperature was more successful at 65° F. than at any lower or higher level (tables 15 and 16; fig. 27). Development was rapid (tables 6 and 15; fig. 28), overlapping of stages was practically nil, mortality was low, and abnormalities were few. The details offered in this section are based on an experiment conducted under refrigeration. Essentially the same results were obtained in experiments conducted with heat and the circulator.

Stages did not overlap at 65° F. with the exception of the overlap of stages 4 and 5 and of stages 13 and 14.

The percentage of dead embryos was low in most samples but the day-to-day variation of that percentage (fig. 27) was highly erratic and for several days the percentage exceeded that of the terminal sample taken on the 19th day. No verifiable explanation can be offered for this behavior of the percentages, but it is possible that dead eggs were not distributed evenly through the experimental lot and that certain samples happened to be taken from points at which dead embryos were concentrated. The 78-percent survival to the end of the experiment (only 22 percent dead in the terminal sample) was by far the highest

TABLE 15.—*Living and dead embryos per sample and their stage of development at 65° F. (18.4° C.)*

Sample	Hours	Number dead	Stage of living embryos							
			1	2	3	4	5	6	7	8
1	½		195							
2	1		330							
3	2		112	1						
4	3			279						
5	4			224						
6	5			433						
7	6	2		272						
8	7	1		287						
9	8	2		11	500					
10	9	3			169					
11	10	5			376	1				
12	11	3			51	307				
13	12	3				315				
14	13	12				303	62			
15	14	11				36	381			
16	15	9				4	274			
17	16	14						346		
18	17	14						329		
19	18	4						97		
20	19	14						19	324	
21	24	11							245	
22	28	24								476
23	29	19								361
24	30	23								329
25	31	7								334

Sample	Hours	Number dead	Stage of living embryos						
			8	9	10	11	12	13	14
26	32	10	208						
27	45	9	218						
28	53	22	227						
29	56	81	247						
30	64	91	7	189					
31	69	82		304					
32	80	65		337					
33	93	65		365					
34	104	231		96	78				
35	116	181			161				
36	128	113			99	194			
37	142	284				61			
38	152	80				74	258		
39	164	6					139		
40	176	118					231		
41	188	179					135		
42	200	51					64	171	
43	212	107						170	
44	224	100						60	
45	236	97						84	
46	248	38						39	43
47	271	3						20	135
48	285	5						10	129
49	296	6						19	205

TABLE 16.—*Mortalities of sea lamprey eggs reared at a constant temperature of 65° F. (18.4° C.)*

[No samples on 15th and 18th days]

Time		Stage span	Number of samples	Number of embryos			Percentage dead
Hours	Days			Alive	Dead	Total	
0–24	1	0–7	21	6,283	108	6,391	2
25–48	2	8	6	1,926	92	2,018	5
49–72	3	8–9	4	974	276	1,250	22
73–96	4	9	2	702	130	832	16
97–120	5	9–10	2	335	412	747	55
121–144	6	10–11	2	354	397	751	53
145–168	7	11–12	2	471	86	557	15
169–192	8	12	2	366	297	663	45
193–216	9	12–13	2	405	158	563	28
217–240	10	13	2	144	197	341	58
241–264	11	13–14	1			120	
265–288	12	13–14	2			302	
289–312	13	13–15	2			384	
313–336	14	14–15	2			271	
361–384	16	15–16	1			31	
385–408	17	15–17	2			117	
433–456	19	17	1			114	
438 [1]	19	14–17		11,918	3,355	15,273	22

[1] Terminal sample.

among the experiments and represents unusually good results for the artificial rearing of fish eggs of any kind.

Abnormalities were extremely few, approximately 2 to 5 percent, mostly in the last samples. A few specimens had enlarged pericardia with a straight, tubular heart, and several had yolk separation and the associated hydrocoelus gut. One "twin" embryo was seen. These twins, in early stage 12, possessed a common but somewhat enlarged blastopore (fig. 29).

Development at 70° F.

The rate of development at 70° F. was only slightly accelerated over that at 65° F., in fact

FIGURE 29.—Posterolateral view of twin embryos, showing the common, but enlarged, blastopore.

embryos reared at 70° F. reached stages 2 and 4 later than did those reared at 65° F. (table 6; fig. 28), and the limited overlap of stages characterized embryos at both temperatures. More pronounced and more significant were the increase of mortality (tables 17 and 18; fig. 27) and the greater number of abnormalities at 70° F. as compared with the "optimum" of 65° F.

At 70° F. as in the 65° test, the overlapping of stages was limited to a slight overlap between stages 11 and 12 and a somewhat greater one between stages 13 and 14 (tables 6 and 17). Elsewhere, the progression from one stage into the next was precise. Stage 8 was prolonged over 11 samples covering 32 hours, as against 2 to 3 for all other early stages.

TABLE 17.—*Living and dead embryos per sample and their stage of development at 70° F. (21.1° C.)*

nple	Hours	Number dead	Stage of living embryos							
			1	2	3	4	5	6	7	8
-----	1	----------	277	-----	-----	-----	-----	-----	-----	-----
-----	3	----------	195	-----	-----	-----	-----	-----	-----	-----
-----	5	----------	-----	130	-----	-----	-----	-----	-----	-----
-----	7	50	-----	84	74	-----	-----	-----	-----	-----
-----	9	8	-----	-----	121	-----	-----	-----	-----	-----
-----	11	13	-----	-----	5	124	-----	-----	-----	-----
-----	13	43	-----	-----	-----	56	93	-----	-----	-----
-----	15	31	-----	-----	-----	-----	58	69	-----	-----
-----	17	39	-----	-----	-----	-----	-----	45	96	-----
-----	19	42	-----	-----	-----	-----	-----	-----	76	71
-----	21	41	-----	-----	-----	-----	-----	-----	39	81
-----	23	26	-----	-----	-----	-----	-----	-----	-----	151
-----	25	40	-----	-----	-----	-----	-----	-----	-----	113
-----	27	34	-----	-----	-----	-----	-----	-----	-----	122
-----	31	38	-----	-----	-----	-----	-----	-----	-----	138
-----	35	28	-----	-----	-----	-----	-----	-----	-----	123
-----	39	38	-----	-----	-----	-----	-----	-----	-----	135
-----	43	16	-----	-----	-----	-----	-----	-----	-----	71

Sample	Hours	Number dead	Stage of living embryos						
			8	9	10	11	12	13	14
19-----	47	45	99	24	-----	-----	-----	-----	-----
20-----	51	38	48	61	-----	-----	-----	-----	-----
21-----	58	65	-----	122	-----	-----	-----	-----	-----
22-----	71	92	-----	160	-----	-----	-----	-----	-----
23-----	82	89	-----	62	31	-----	-----	-----	-----
24-----	95	325	-----	-----	13	-----	-----	-----	-----
25-----	103	221	-----	-----	58	61	-----	-----	-----
26-----	116	258	-----	-----	-----	41	6	-----	-----
27-----	127	44	-----	-----	-----	12	53	-----	-----
28-----	139	206	-----	-----	-----	-----	30	-----	-----
29-----	151	139	-----	-----	-----	-----	5	35	-----
30-----	163	195	-----	-----	-----	-----	-----	87	-----
31-----	175	64	-----	-----	-----	-----	-----	33	13
32-----	187	48	-----	-----	-----	-----	-----	15	20
33-----	199	25	-----	-----	-----	-----	-----	18	26
34-----	211	46	-----	-----	-----	-----	-----	8	44
35-----	225	24	-----	-----	-----	-----	-----	1	32
36-----	235	24	-----	-----	-----	-----	-----	1	41

BLE 18.—*Mortalities of sea lamprey eggs reared at a constant temperature of 70° F. (21.1° C.)*

[No sample on 24th day]

Time		Stage span	Number of samples	Number of embryos			Percentage dead
Hours	Days			Alive	Dead	Total	
4----------	1	0–8	12	1,845	293	2,138	14
48----------	2	8–9	7	825	239	1,064	22
72----------	3	8–9	3	391	195	586	33
96----------	4	9–10	2	106	414	520	80
120----------	5	10–12	2	166	479	645	74
-144----------	6	11–12	2	95	250	345	72
-168----------	7	12–13	2	127	334	461	72
-192----------	8	13–14	2	--------	--------	193	--------
-216----------	9	13–14	2	--------	--------	167	--------
-240----------	10	13–15	2	--------	--------	160	--------
-264----------	11	14–15	2	--------	--------	149	--------
-288----------	12	15	2	--------	--------	124	--------
-312----------	13	15–16	2	--------	--------	118	--------
-336----------	14	16	2	--------	--------	32	--------
-360----------	15	16–17	2	--------	--------	69	--------
-384----------	16	17	2	--------	--------	91	--------
-408----------	17	17	2	--------	--------	98	--------
-432----------	18	17	2	--------	--------	21	--------
-456----------	19	17	1	--------	--------	68	--------
-480----------	20	17	1	--------	--------	109	--------
-504----------	21	17	2	--------	--------	65	--------
-528----------	22	17	2	--------	--------	59	--------
-552----------	23	17	1	--------	--------	28	--------
-600----------	25	17	1	--------	--------	29	--------
-624----------	26	17	1	--------	--------	5	--------
-648----------	27	17	1	--------	--------	50	--------
1----------	27	17	1	528	10,291	10,819	95

Terminal sample.

The percentage of dead embryos in the samples rose rapidly to 80 percent on the fourth day and continued above 70 percent through the seventh day after which hatching made the further samples undependable as measured at mortality (table 18; fig. 27). The percentage of embryos dead in the 70°-terminal sample (95 percent) indicates final production a little less than half that at 60° F. (88 percent dead in 60° terminal sample).

Abnormalities in this experiment were relatively few (15 to 20 percent of the prolarvae), but more numerous than at 65° F. The abnormalities were similar to those listed for the 60° F. test, especially with respect to body shape.

Development at 75° F.

No viable, burrowing larvae were produced at a constant temperature of 75° F. Heavy mortality appeared early and persisted throughout development (tables 19 and 20; fig. 27) with the result that few embryos survived even to hatching. With the exception of stages 2 and 3 the various

TABLE 19.—*Living and dead embryos per sample and their stage of development at 75° F. (23.9° C.)*

mple	Hours	Number dead	Stage of living embryos							
			1	2	3	4	5	6	7	8
-----	1	----------	129	-----	-----	-----	-----	-----	-----	-----
-----	3	25	200	-----	-----	-----	-----	-----	-----	-----
-----	5	25	-----	113	-----	-----	-----	-----	-----	-----
-----	7	102	-----	-----	57	1	-----	-----	-----	-----
-----	9	186	-----	-----	-----	40	2	-----	-----	-----
-----	11	71	-----	-----	-----	12	148	-----	-----	-----
-----	13	81	-----	-----	-----	-----	113	40	-----	-----
-----	15	88	-----	-----	-----	-----	-----	133	81	-----
-----	17	85	-----	-----	-----	-----	-----	-----	177	-----
-----	19	172	-----	-----	-----	-----	-----	-----	-----	175
-----	21	191	-----	-----	-----	-----	-----	-----	-----	102
-----	23	103	-----	-----	-----	-----	-----	-----	-----	62
-----	25	53	-----	-----	-----	-----	-----	-----	-----	89
-----	27	103	-----	-----	-----	-----	-----	-----	-----	80
-----	31	163	-----	-----	-----	-----	-----	-----	-----	89
-----	35	105	-----	-----	-----	-----	-----	-----	-----	202

Sample	Hours	Number dead	Stage of living embryos						
			8	9	10	11	12	13	14
17-----	39	54	69	-----	-----	-----	-----	-----	-----
18-----	43	60	17	36	-----	-----	-----	-----	-----
19-----	47	207	-----	-----	-----	-----	-----	-----	-----
20-----	51	153	-----	54	-----	-----	-----	-----	-----
21-----	58	268	-----	81	-----	-----	-----	-----	-----
22-----	71	260	-----	45	4	-----	-----	-----	-----
23-----	82	322	-----	8	23	-----	-----	-----	-----
24-----	95	188	-----	-----	21	27	-----	-----	-----
25-----	103	287	-----	-----	4	26	3	-----	-----
26-----	116	124	-----	-----	-----	14	37	-----	-----
27-----	127	202	-----	-----	-----	-----	13	-----	-----
28-----	139	210	-----	-----	-----	-----	7	-----	-----
29-----	151	265	-----	-----	-----	-----	-----	14	-----
30-----	163	176	-----	-----	-----	-----	-----	8	-----
31-----	175	106	-----	-----	-----	-----	-----	1	11

TABLE 20.—*Mortalities of sea lamprey eggs reared at a constant temperature of 75° F. (23.9° C.)*

Time		Stage span	Number of samples	Number of embryos			Percentage dead
Hours	Days			Alive	Dead	Total	
0–24	1	0–8	12	1,585	1,129	2,714	42
25–48	2	8–9	7	582	745	1,327	56
49–72	3	9–10	3	184	681	865	79
73–96	4	9–11	2	79	510	589	87
97–120	5	10–12	2	84	411	495	83
121–144	6	12	2	20	412	432	95
145–168	7	13	2	22	441	463	95
169–192	8	13–14	2	------	------	325	------
193–216	9	14–15	2	------	------	355	------
217–240	10	15–16	2	------	------	265	------
241–264	11	16	2	------	------	94	------
265–288	12	16	1	------	------	27	------
307 [1]	13	------	1	------	5,600	5,600	100

[1] Terminal sample.

stages were reached more rapidly than at lower temperatures and the overlap of stages was limited (tables 6 and 19; fig. 28).

Transition from one stage into the next was rather precise, as in the two preceding experiments. No stage overlapped another in more than 2 samples (tables 6 and 19). Stage 8 was projected over 9 samples covering 24 hours as contrasted with 2 or 3 for each of the earlier stages.

Substantial mortality appeared early at this temperature, especially between stages 0 and 8. The percentage of dead embryos had reached 95 percent on the seventh day, and the terminal sample did not include any live embryos. Production at this temperature was nil, despite the survival of some individuals as far as stage 16.

It is conceivable that stage 17 possibly could have been reached since the experiment was terminated when the supply of stage 16 prolarvae was exhausted. Were it possible to have allowed development to proceed without sampling, the probability of stage 17 being reached would have been improved.

Abnormalities occurred in 35 to 40 percent of the prolarvae. These abnormalities were similar to those already listed except that deformed specimens were especially numerous.

Development at 77.5° F.

A constant temperature of 77.5° F. was decidedly above the maximum at which sea lamprey eggs could develop successfully. Mortality was so great that all embryos were dead after 13 hours and none developed beyond stage 6, 32 cells (tables 6, 21, and 22; fig. 27).

Stage 1 was taken in the 1- to 4-hour samples, stage 2 in the 4- and 6-hour samples, and stage 3 in the 6- to 10-hour samples. Only 4 embryos developed beyond stage 3, and only 3 (stages 5 and 6) were still alive in the sample taken at 13 hours. All were dead in samples from 14 hours to the termination of the experiment at 24 hours. Some stages were reached earlier at 77.5° F. than at 75° F. (stages 2 and 3), but others were attained later at the higher temperatures (stages 4 and 5). See table 6 and fig. 28.

TABLE 21.—*Living and dead embryos per sample and their stage of development at 77.5° F. (25.3° C.)*

Sample	Hours	Number dead	Stage of living embryos					
			1	2	3	4	5	6
1	1	------	153	------	------	------	------	------
2	3	1	67	------	------	------	------	------
3	4	10	29	36	------	------	------	------
4	6	58	------	32	4	------	------	------
5	8	20	------	------	31	------	------	------
6	10	64	------	------	8	1	------	------
7	13	70	------	------	------	------	1	------

TABLE 22.—*Mortalities of sea lamprey eggs reared at a constant temperature of 77.5° F. (25.3° C.)*

Time		Stage span	Number of samples	Number of embryos			Percentage dead [1]
Hours	Days			Alive	Dead	Total	
0–24	1	0–6	13	364	708	1,072	68

[1] All embryos (485) dead in the 6 samples taken after 13 hours.

Development at 80° F.

Development was brief and erratic at a constant temperature of 80° F. A few embryos reached the 32-cell stage (stage 6) but most were dead long before that stage was reached (tables 6, 23, and 24; figs. 27 and 28).

No signs of cleavage had appeared at the end of the first 3 hours but at the fifth hour practically all of the eggs had begun to dimple, the initiation of first cleavage and stage 2. By the ninth hour all eggs had completed first cleavage and many had started the second cleavage (start of stage 3). At the 11th hour the pattern of cleavage had become rather erratic, since the second cleavage furrow often began while the first was less than half-completed. At times the first cleavage furrow seemed to regress.

Mortality which was slight in the first 2 samples had risen to about 35 percent in the seventh hour. All embryos were dead in the sample at 13 hours, but the next sample at 15 hours contained eggs

at had reached stage 6 (32 cells) but had begun vacuolate. All subsequent samples had only ad embryos.

BLE 23.—*Living and dead embryos per sample and their stage of development at 80° F. (26.6° C.)*

Sample	Hours	Number dead	Stage of living embryos					
			1	2	3	4	5	6
----------	3	6	131	------	------	------	------	------
----------	5	7	107	16	------	------	------	------
----------	7	85	35	131	------	------	------	------
----------	9	124	------	11	55	------	------	------
----------	11	149	------	------	31	------	------	------
----------	13	165	------	------	------	------	------	------
----------	15	303	------	------	------	------	------	2

BLE 24.—*Mortalities of sea lamprey eggs reared at a constant temperature of 80° F. (26.6° C.)*

Time		Stage span	Number of samples	Number of embryos			Percentage dead [1]
Hours	Days			Alive	Dead	Total	
4----------	1	0–6	11	519	1,753	2,272	77

Only 2 live embryos were taken after 11 hours and all (914) were dead r 15 hours.

SIGNIFICANCE OF OBSERVATIONS

fect of temperature on development

The most significant result of the experimental aring of sea lamprey eggs at 10 different constant mperatures (ranging from a minimum of 45° to a maximum of 80° F.) was the clear demon-ration that successful development through to e production of viable burrowing larvae was ssible only within a relatively narrow range. o live larvae were produced at any temperature low 60° F. or above 70° F. Further evidence the extreme sensitivity of sea lamprey eggs to mperature comes from the much lower survival 60° F. (12 percent), and 70° F. (5 percent), an occurs at the "optimum" temperature of 65° (78 percent). It is to be regretted that experi-ents were not made at 62.5° F. and 67.5° F. to fine more clearly the trends within the 60°–70° nge, but no further time was available when e importance of tests at these two intermediate mperatures became obvious.

Mortality was so heavy at the highest and lowest mperatures that all eggs had died before devel-ment had proceeded beyond very early stages. general, the highest stage reached increased as e temperature approached the "successful" levels of 60°–70° F. This relationship is brought out by the following listing:

Temperature (F.)	*Highest stage reached*
45°----------	3 (4 cells)
50°----------	9 (gastrula)
52.5°----------	12 (head)
55°----------	13 (prehatching)
60°----------	17 (burrowing)
65°----------	17 (burrowing)
70°----------	17 (burrowing)
75°----------	16 (gill clefts)
77.5°----------	6 (32 cells)
80°----------	6 (32 cells)

Developmental abnormalities were least plentiful at 65° F. and increased as the temperature deviated from that value in either direction. In some tests the incidence was high, and the abnormalities (described briefly in the accounts of the experiments) involved monstrous distortions of the embryos.

In general, developmental rate (notably, the time required to reach the various stages) became faster, lengths of stages became shorter, and overlap between stages was lessened as temperature increased. Some of the exceptions to this statement no doubt represent the random variability of the data. Others, as for example, the seemingly depressing effect of the highest temperatures on the rate of development in the early stages may reflect a real cause-and-effect relationship.

The clear demonstration in the present studies that sea lamprey eggs are capable of full and normal development only within a relatively narrow temperature range brings out the great importance of controlling temperature at the correct level in developmental studies and experimental research. Consideration of the proper temperature had little place, nevertheless, in past studies of sea lamprey eggs. Authors failed to state the temperatures at which the eggs were reared or reared them at levels at which full, normal development could not be expected. Shipley (1885) did not state the temperature at which his sea lamprey embryos developed and McClure (1893) reared his embryos at 6°–7° C. (42.8°–44.6° F.). Damas (1948) mentioned temperatures of 12° and 18° C. (53.6° and 64.4° F.) on the development of *Lampetra* (it is not to be assumed, of course, that the effects of temperature on development are the same for *Petromyzon* and *Lampetra*, but certain parallels must be considered highly probable).

Shipley's account of the persistence of the blastopore and his statement that invagination took place at 130 hours suggest that embryos of the lamprey developed at a temperature of about 55° F. It appears then that neither he nor McClure conducted their experiments within the range at which normal development could be expected. If the relation of temperature to development in *Lampetra* is similar to that in *Petromyzon*, Damas' experiments at 18° C. (64.4° F.) should have been at nearly the optimum temperature, but those conducted 12° C. (53.6° F.) were well below the optimum. Thus, certain of the abnormalities (most of them duplicated in the present study, particularly at the higher and lower temperatures) that he interpreted as the effect of light intensity may actually have been caused by temperature.

Although the findings of the present experiments offer the strongest evidence that unsuitable temperatures may account for the failure of certain apparently suitable streams to produce larval sea lampreys, a too close application of the results to problems in nature is not advisable. The sea lamprey eggs were reared at constant temperatures in this study, whereas the temperatures in natural streams are subject to diurnal fluctuations, to substantial short-term increases and decreases along with changes of weather, and finally to a longer term, seasonal, upward trend as development proceeds. These fluctuations may have a profound effect on the tolerance of the developing egg. Temperature surely is an important, sometimes a critical, factor in the production of viable larvae in nature, but a good understanding of its operation would require controlled investigations in which eggs develop under fluctuating temperatures which are made to vary much as they do in natural streams.

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